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THE PRODUCTION OF ASEXUAL SPORES BY PLEUROTUS CORTICATUS [1]

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(WITH PLATES 26–31)

Asexually produced spores of several types, conidia, oidia, and chlamydospores, have been described for many species of the Agaricales, but they and the structures upon which they are formed are usually inconspicuous. Such spores have been described as being formed by segmentation of single hypha, or upon the tips of specialized branches of individual hypha. They are usually formed only in culture and can be found only by close microscopic examination of the vegetative mycelium. Compound fruiting bodies, such as coremia, acervuli, and pycnidia, apparently have not been described for the Agaricales.

While making a study of fire and decay damage in the bottomland hardwood region of northeastern Louisiana in 1931, the writer isolated an agaric which formed tall, white coremia, capped with glistening masses of black conidia. The fungus was isolated from the incipient decay in a red gum tree (*Liquidambar styraciflua*) which had been fire-scarred 15 years previously. The

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[2] The writer wishes to express his indebtedness to Dr. E. C. Stakman, under whose direction this work was done, and to Dr. L. O. Overholts, Department of Botany, Pennsylvania State College, for identifying several sporophores sent him.

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production in culture of these striking compound sporophores and asexual spores by a wood-destroying Basidiomycete stimulated study of the fungus, and especially of the accessory spores.

Fortunately, small sporophores were formed in culture when the fungus was grown on 4.0 per cent malt extract agar. The production of these small, white, generally imperfect sporophores with eccentric stipes and decurrent gills suggested that the fungus was possibly a *Panus* or a *Pleurotus*. The following spring a *Pleurotus*-like sporophore was collected near Greenville, Mississippi, and, while it was drying, white coremia and black conidial heads developed on the gills, pileus, and stipe. Tissue cultures made from this sporophore also produced an abundance of coremia and black conidial heads similar to those formed upon the sporophore and those found in the culture made from the decayed wood the previous season.

This sporophore, which unfortunately was in poor condition, was sent to Dr. L. O. Overholts. On the basis of the unusual elongate-cylindrical basidiospores, 12.5–19 by 4.3–6.0 μ, and other recognizable characteristics, he tentatively identified it as *Pleurotus corticatus*. In the past year the fungus has been grown on a number of media that induce fruiting of many stipitate agarics, and several fairly large sporophores have formed. Although they were no doubt abnormal in some respects, they resembled closely the original sporophore. From the original sporophore and those formed in culture the following description may be given for the fungus.

Pileus compact, firm when dry, convex to expanded, 4–8 cm. broad, dull white at first, becoming grey to yellow-brown with age, finely floccose at first, becoming almost squamose toward the stipe at maturity, solitary or occasionally cespitose. Flesh thick, white, brittle. Gills white, becoming yellow with age, decurrent, occasionally anastomosing toward the stipe, edge entire, rather broad in front, tapering toward the base. Stem 4–8 cm. long, lateral in most specimens, solid, slightly pubescent, tapering considerably. Veil rarely formed on sporophores developed in culture, fugacious, rarely leaving an annulus; when present it consists of small scale-like remnants closely appressed to the stipe. There is no evidence of a veil on the original sporophore. Basidiospores cylindric-elongate, 12.5–19 by 4.3–6.0 μ, smooth, pure-white in mass. Sporophores becoming covered

with tall white coremia, bearing glistening black drops of conidia at their tips, when placed in a humid chamber or when dried slowly.

This description agrees closely with the one given for *Armillaria corticata* Fries-Pat., by Kauffman (**4**). Kauffman discarded the old subdivision of the genus *Pleurotus*, set up by Fries to include the two species possessing a fugacious veil, *Pleurotus corticatus* and *Pleurotus dryinus*, and has transferred these species to the genus *Armillaria*. The only really significant difference between the two species is in spore size, those of *Armillaria* (*Pleurotus*) *corticata* being larger than those of *Armillaria* (*Pleurotus*) *dryina*, which are 9–10 by 4–4.5 μ. Atkinson (**1**) considers *Pleurotus corticatus* merely a form of *P. dryinus*. Murrill's description (**5**) for *Pleurotus dimidiatus* (Schaeff.) Murrill also fits my fungus quite well, the spore dimensions he gives being somewhat smaller, corresponding with those given for *Armillaria dryina* by Kauffman.

SPOROPHORE PRODUCTION

The fungus grows well upon malt extract agar, producing a luxuriant mycelium, an abundance of coremia, aborted sporophores, and occasionally a sporophore with gills and pileus. But nutrient agar, even in large quantities, does not induce the degree of vegetative development prerequisite to sporophore production by the larger stipitate agarics. To obtain sufficient vegetative growth it is necessary to have a medium that is porous and contains an abundance of food material, as pointed out by Etter (**3**). Such a medium was obtained by adding a 4.0 per cent malt extract solution to basswood sawdust in quart jars. Six weeks to two months after inoculation the mycelium usually filled the jar and had begun to form sporophore primordia. The quart jars were then placed under bell jars and the caps removed. Abortive sporophores, such as those shown in plate 26, figures 1 and 2, were commonly formed, but when the conditions were exactly right, large, apparently normal sporophores, such as the one shown in plate 27, figure 1 and 2, were formed. Such sporophores produced an abundance of basidiospores. From the numerous tests that have been made upon many media under a large variety of condi-

tions, the requirements for sporophore production by *Pleurotus corticatus* appear to be the following: A porous medium containing an abundance of sugars, particularly maltose; rather high temperatures, 27 degrees C., being the optimum for vegetative growth. Abundant vegetative growth is a necessary preliminary to sporophore production. Once the necessary vegetative growth has been made at the high temperature, sporophore production will take place at a lower temperature. Sunlight or diffuse light is necessary for sporophore production, although vegetative growth is most rapid in the dark.

BASIDIOSPORES

The basidia of the fungus are four-spored. The basidiospores are 12.5–19.0 μ by 4.3–6.0 μ and contain a single nucleus (PLATE 30, FIG. 1). On germination a haploid mycelium is produced. This differs from the binucleate mycelium in rate of growth and in the absence of clamp connections, but, like the dicaryotic mycelium, it forms coremia and conidia.

It has been very difficult to induce the basidiospores to germinate. Although a large number of media and stimulatory substances have been tried under many different conditions, it has been possible to obtain but five haploid lines. The conditions required for germination have not yet been determined.

From the results of mating experiments with the five haploid lines and diploidization tests with the parent dicaryotic mycelium and these haploid lines, it is evident that the fungus is heterothallic. Further work upon the sexuality and nuclear phenomena of the fungus is now under way and will be reported on later.

CONIDIA

In addition to the conidia which are formed in the tall coremia on both the binucleate and haploid mycelia, simple conidia also are produced, single spores being formed on short conidiophores. Therefore, four types of conidia are formed: binucleate conidia formed in coremia on the dicaryotic mycelium; simple, binucleate conidia borne singly at the tips of short hyphal branches on the dicaryotic mycelium; uninucleate conidia formed in coremia on the haploid mycelium; and simple, uninucleate conidia borne singly at the tips of short conidiophores on the haploid mycelium.

Binucleate conidia in coremia. Binucleate conidia are formed in coremia on perfect sporophores, abortive or abnormal sporophores, and upon the dicaryotic mycelium when the fungus is grown on an agar medium.

When sporophores are placed in a humid chamber or are dried slowly, they soon become covered with tall white coremia, each capped by a black glistening drop of conidia (PLATE 27, FIG. 3 AND 4, AND PLATE 28). The coremia begin as small tufts of white mycelium upon the stipe and gills, these increasing in diameter and height until in some cases they may be 2 cms. tall and 0.5 cm. in diameter. The coremia formed upon the gills are normally much smaller than those on the stipe. After 4 days in a damp chamber spore formation commences and the coremial heads become black and glistening. After two weeks in a humid chamber the sporophores have shrunken somewhat, as seen in plate 27, figure 4, and are covered with coremia. The sporophores show a remarkable resistance to decay by molds and bacteria, for even after 2 weeks in such a chamber the sporophores are not molded or decomposed badly.

Coremia and conidia of the same type as are formed upon normal sporophores are formed in culture on abnormal or aborted sporophores, which are produced abundantly on any medium that permits vegetative growth. Plate 26, figure 1, and plate 29, figure 1, illustrate this type of sporophore formation. The aborted sporophores are often completely covered and blackened by such coremia (PLATE 29, FIG. 2).

Coremia are also formed directly on the binucleate vegetative mycelium when the fungus is grown upon nutrient media. The coremia have essentially the same structure and are of about the same size as those formed upon normal and abnormal sporophores (PLATE 30, FIG. 1 AND 2).

The coremia formed on the binucleate mycelium are composed of tightly compacted masses of hyphae which have prominent clamp connections at every cross wall. At the tips of the coremia the mycelium is less tightly compacted, the free ends of the hyphae breaking up in a basipetal manner into chains of conidia. The structure of the coremia and method of spore formation are illustrated in plate 31, figures 4 and 6. The clamp connection

becomes a part of the spore formed beneath the septum on which the clamp occurs. The terminal spores of a chain are usually rounded and mature. Below these are others with distinct beak-like projections, the remnants of the old clamp connections. Deeper in the head are still more immature spores with clamp connections still attached and distinct. The conidia vary greatly in size, the spores in a single head sometimes ranging from 9.0 to 27.0 μ in length and from 4.0 to 8.5 μ in width. The walls of the spores are yellow-brown, appearing black in masses and when wet. The liquid in which they are formed is colorless, the drop at the tip of the coremia obtaining its black color from the mass of dark-walled spores. The nuclei of the conidia are easily stained with iron-alum-haematoxylin, there usually being two nuclei in each spore. A few spores have been found, however, which contained but a single nucleus. Whether or not these are actually haploid conidia formed upon the dicaryotic mycelium, as described for *Pholiota aurivella* by Martens and Vandendries (**6**), is still uncertain. All the conidia that have been germinated have given rise to binucleate mycelia with prominent clamp connections at every cross wall. The number of conidia formed in a single head is astonishing. The spores from a single head were washed onto a slide, evenly distributed, and counts made on small sample areas. On the basis of such counts a single head 1.0 mm. in diameter was found to contain about 10,000 spores. Some of the larger heads, which may be 7 mm. in diameter, probably contain over a million spores.

Simple binucleate conidia. In addition to the binucleate conidia in coremia, the fungus forms a second type of binucleate conidia, single binucleate spores borne singly at the tips of small conidiophores (PLATE 31, FIG. 2). These spores appear to be formed only when the fungus is growing on a medium poor in nutrients or when the fungus is grown under adverse temperature and moisture conditions. The spores are small, round, and vary from 2.5 to 4.2 μ in diameter.

Uninucleate conidia in coremia. The haploid mycelium of *Pleurotus corticatus*, resulting from a single basidiospore, differs from the dicaryotic mycelium in rate of growth, the growth of the haploid lines being about one-half as fast as that of the dicaryotic

mycelium; in the absence of clamp connections; and in the lack of sporophore formation. The haploid mycelium resembles the dicaryotic in that it forms conidia in coremia and also simple conidia. The coremia formed on the haploid mycelium are usually smaller than those on the dicaryotic mycelium but have the same general structure (PLATES 30 AND 31). The structure of the haploid coremia is shown in plate 31, figure 7, and the method of spore formation in figure 5. The uninucleate conidia vary greatly in size; the spores from a single head may vary from 4.5 to 15.0 μ in length and from 4.0 to 7.0 μ in width. The uninucleate conidia are thus considerably smaller than the binucleate conidia. Upon germination these uninucleate conidia always give rise to mycelia of the same sex as those upon which they were produced. Although the conidial heads formed on the haploid mycelium are smaller than those formed on the dicaryotic mycelium, the spores produced are equally numerous because they are much smaller. The uninucleate conidia are produced in basipetal succession, have yellow-brown walls, and are borne in a drop of a thin colorless liquid. From the appearance of these spores and the sticky fluid in which they are formed it would seem that they probably are disseminated by insects in nature, much like the haploid oidia of *Coprinus Lagopus* described by Brodie (**2**).

Simple uninucleate conidia. Like the dicaryotic mycelium the haploid mycelium forms simple conidia, borne singly upon short conidiophores, when grown under adverse conditions. These spores are always uninucleate, are small, 1.7–3.5 μ, and have yellow-brown walls. This difference in size of the simple binucleate conidia and the simple uninucleate conidia constitutes another constant difference between the haploid and dicaryotic mycelia. The way these spores are borne on the haploid mycelium is shown in plate 31, figure 3.

SUMMARY

1. The production of asexual spores by *Pleurotus corticatus* Fries is described.

2. The formation of conidia on coremia is described for an agaric for the first time, as far as the writer is aware.

3. Binucleate conidia are formed in coremia on normal sporophores, if the sporophores are placed in a humid chamber while still fresh; upon abnormal or aborted sporophores; and upon the dicaryotic vegetative mycelium when the fungus is grown upon a nutrient medium.

4. Simple binucleate conidia are formed singly at the tips of small conidiophores on the dicaryotic mycelium when the fungus is grown under adverse conditions.

5. Uninucleate conidia are formed on coremia on the haploid mycelia derived from single basidiospores.

6. Simple uninucleate conidia are formed singly at the tips of small conidiophores on the haploid mycelium when the fungus is grown under adverse conditions.

7. Fairly large sporophores of the fungus may be produced on a medium composed of basswood sawdust and malt extract. Basidiospores from such sporophores do not germinate very readily.

8. From tests with the few haploid lines obtained it has been established that the fungus is heterothallic.

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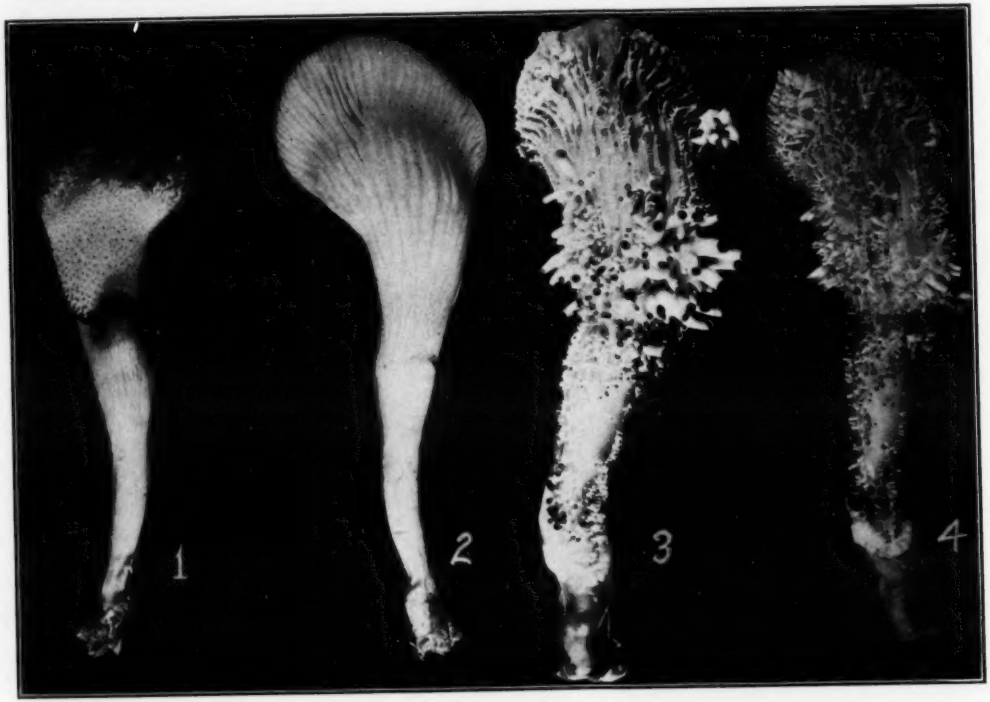
LITERATURE CITED

1. **Atkinson, G. F.** Studies of American fungi. Mushrooms, edible, poisonous, etc. 1901.
2. **Brodie, H. J.** The oidia of *Coprinus Lagopus* and their relation with insects. Ann. Bot. **45**: 315–344. 1931.
3. **Etter, B. E.** New media for developing sporophores of wood-rot fungi. Mycologia **21**: 197–203. 1929.
4. **Kauffman, C. H.** The Agaricaceae of Michigan. Mich. Geol. and Biol. Survey, Pub. 26, Biol. Series 5. 1918.
5. **Murrill, W. A.** Agaricaceae. N. Am. Fl. **10**: 35. 1914.
6. **Martens, P., & R. Vandendries.** Le Cycle Conidien Haploide et Diploide chez *Pholiota aurivella*. Extrait de "La Cellule" **41**, fascicule 4, 337–388. 1933.

EXPLANATION OF PLATES

PLATE 26

Fig. 1, abortive primary and secondary sporophores of *Pleurotus corticatus* produced on a mixture of basswood sawdust and malt extract but under light and humidity conditions that were unfavorable for the fungus; 2, abnormal sporophores of *Pleurotus corticatus* formed upon a mixture of basswood sawdust and malt extract when subjected to too intense light which causes the entire pileus to become inverted.



PLEUROTUS CORTICATUS

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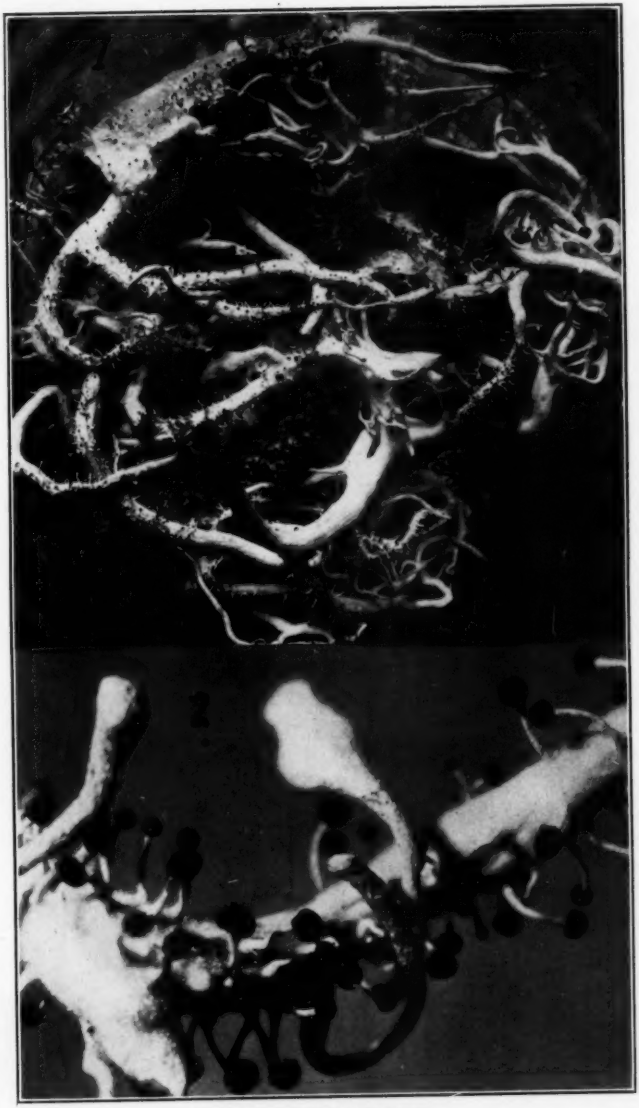

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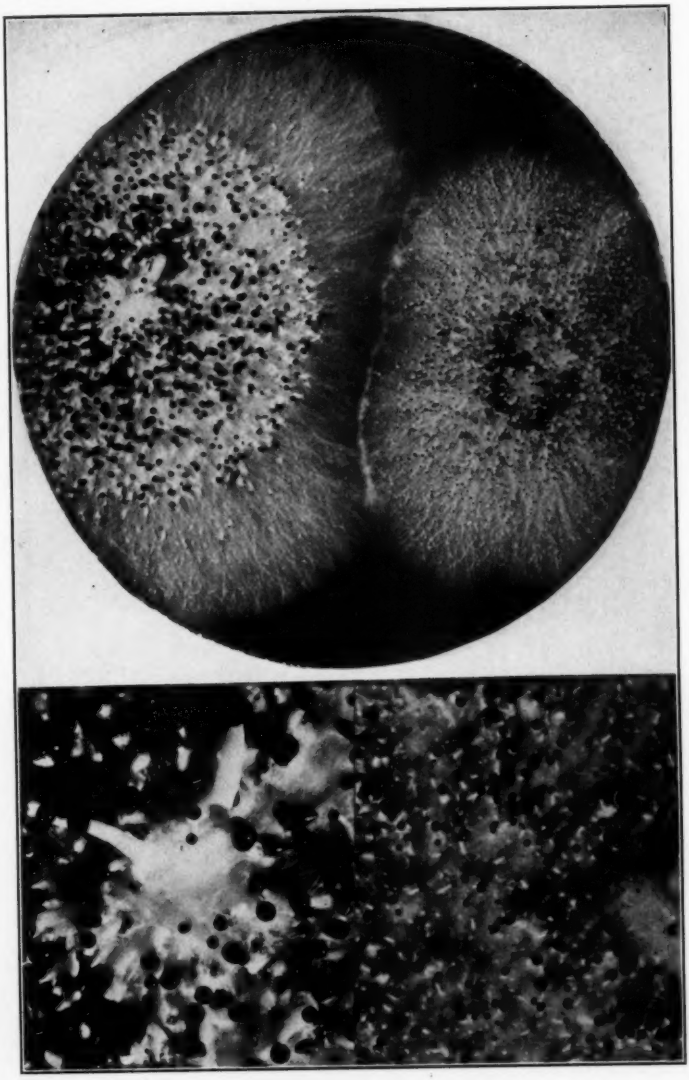

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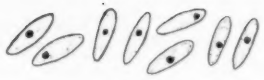

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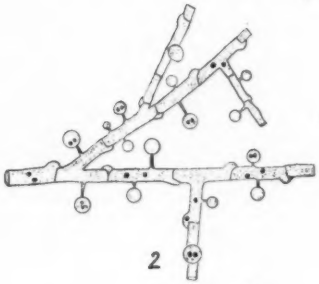

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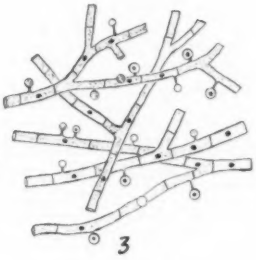

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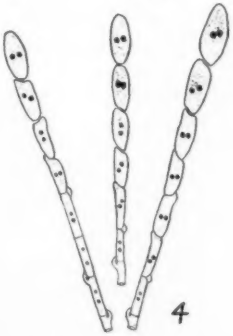

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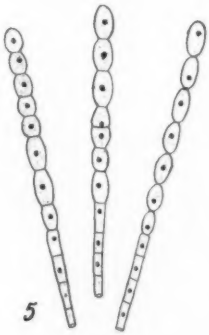

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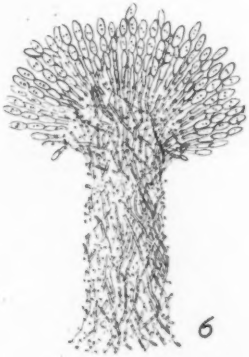

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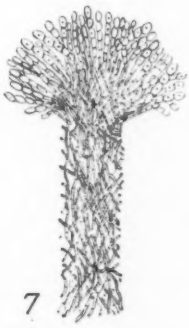

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PLEUROTUS CORTICATUS

PLATE 27

A sporophore of *Pleurotus corticatus* formed in pure culture upon a mixture of basswood sawdust and 5 per cent malt extract. Natural size. Fig. 1, top view of the sporophore showing the long eccentric stipe and the dark color of the pileus; 2, lower surface of the sporophore showing the decurrent gills; 3, the same sporophore after 6 days in a chamber in which the humidity was kept high, tall white coremia with glistening black drops of binucleate conidia at their tips having developed on the stipe and gills; 4, the same sporophore after 12 days in the humid chamber, shrunken in size and many more coremia and conidial heads having developed upon the stipe and gills.

PLATE 28

The same sporophore as is shown in plate 27, figure 4, but enlarged to about 1½ times natural size to show the formation of coremia and conidia upon the gills and stipe in greater detail. Some of the abortive secondary sporophores shown in plate 27, figure 3, were broken off when the photo was made.

PLATE 29

Fig. 1, abortive sporophores and coremia upon a mixture of malt extract and basswood sawdust, the abortive sporophores developing first and then covered by the tall white coremia and black conidial heads (a humid atmosphere in the container is necessary for the formation of coremia upon such aborted sporophores), natural size; 2, a single aborted sporophore enlarged about 3 times to show the nature of the coremia and black spore heads which have formed on them. (Note the secondary aborted sporophores mixed with the coremia.)

PLATE 30

Pleurotus corticatus growing upon malt extract agar. Fig. 1, the large colony at the left is dicaryotic, the smaller one at the right haploid, resulting from a single basidiospore (note the difference in rate of growth of haploid and dicaryotic cultures, the dicaryotic mycelium normally growing almost twice as fast as the haploid, also the difference in size of coremial heads upon the dicaryotic and haploid mycelium), natural size; 2, a small section of the dicaryotic culture is shown at the left and a section of the haploid mycelium at the right, both having been enlarged about 3 times, the difference in size of the dicaryotic and haploid coremia brought out very clearly here.

PLATE 31

The spores of *Pleurotus corticatus*. All drawings made with the aid of a camera lucida and the preparations stained with iron-alum haematoxylin. Figures 1–5 approximately × 500. Fig. 1, basidiospores of the fungus obtained from the sporophore shown in plate 27; 2, binucleate conidia formed singly upon the dicaryotic mycelium when it is grown upon malt extract agar; 3, uninucleate conidia formed singly upon the haploid mycelium grown from a single basidiospore; 4, binucleate conidia or oidia from a coremium formed upon the dicaryotic mycelium (note that the clamp connections gradually d is appear as the spores mature, becoming a part of spore beneath the septum); 5, uninucleate conidia or oidia from a coremium formed upon the haploid mycelium (note the extreme variation in size and shape of these spores); 6, section through a coremium formed upon the dicaryotic mycelium, showing the structure of the coremia and the way the binucleate conidia are borne in the heads, about × 200; 7, section through a coremium formed upon the haploid mycelium, about × 200.

PESTALOTIA SPP. ON AUCUBA, CIBOTIUM AND LEUCOTHOË

R. P. White

(WITH PLATE 32)

During the course of investigations upon the pathogenicity of various species of *Pestalotia*, three undescribed species have come to attention. The species on *Aucuba japonica* var. *variegata*, and *Leucothoë Catesbaei* are weak wound parasites. The type on *Aucuba* follows the large black leaf spots caused by *Colletotrichum Pollaccii* Magn. and occurs generally on sun scalded areas. The type on *Leucothoë* follows infections of *Cryptostictus* sp. and *Lophodermium* sp. as well as injuries to the foliage caused by drying during the winter. The species on *Cibotium Schiedei* has been proved a primary pathogene, causing the destruction of frondlets and entire fronds under the conditions of commercial culture as practiced for this fern in greenhouses.

These three species of *Pestalotia* have been examined critically both on their respective hosts and in culture on a variety of standard media.[1] All three forms are considered new species, since they occur on hosts on which species of *Pestalotia* have not previously been described, and are unlike species which occur on related hosts of the same family.

Pestalotia Aucubae sp. nov.

Conidia 5 cellularia fusoideia 23.6–31.5 × 6.9–8.8 μ (plerumque 25.5–29.5 × 6.9–7.8 μ); duae superiores coloratae cellulae obscuriores inferiore olivacea cellula. Superior cellula hyalina turbinata, setae plerumque 3, generaliter erumpentes ex eodem puncto in apice cellulae superioris, late divergentes, 21–51 μ longae, plerumque 29–41 μ. Conidiophoris filiformibus plerumque 7.8–9.8 μ longis. Acervuli 150–300 μ in diam., generaliter epiphilli.

Conidia 5-celled, fusiform, erect or slightly curved, only slightly constricted at septae, 23.6–31.5 μ long (usually 25.5–29.5 μ; ave. 28.1 μ) by 6.9–8.8 μ wide (usually 6.9–7.8 μ; ave. 7.7 μ); colored cells 13.8–19.7 (usually 15.7–17.7 μ; ave. 16.9 μ); the walls and

[1] Thanks are due to Dr. E. F. Guba for critical examinations of the species as well as for suggestions pertaining to their description.

septae dark, the upper two colored cells darker than lower colored cell, becoming umber or darker with age, guttulate; wall between two upper colored cells usually very dark; apical hyaline cell conic, setae commonly 3, rarely 2, 4 or 5, united or arising at the apex of superior cell, rarely arising from different points, widely divergent, slender, 21.7–51.22 μ long (generally 29–41 μ; ave. 37.6 μ); basal cell typically long conic, pedicel filiform 2.0–17.7 μ long (usually 7.8–9.8 μ; ave. 9.5 μ), generally erect. Pustules 150–300 μ in diameter usually epiphyllous, scattered, the contents issuing as coils surrounded by the torn epidermis or as compact cones.

On foliage of *Aucuba japonica* var. *variegata* following infections of *Colletotrichum Pollaccii* Magn. (PLATE 32 *A*) or injuries due to sun scald. A secondary pathogene. Type locality, Rutherford, N. J. August 20, 1932.

In culture heavy white aerial growth is produced on potato dextrose and Sorbaud's potato dextrose, with scant aerial growth on lima bean, bean pod and corn meal agars and none on prune agar. Sporulation is abundant on potato dextrose, lima bean and bean pod agars in both petri dishes and tubes, but scant on Sorbaud's potato dextrose, corn meal and prune agars. When grown on potato dextrose agar the medium becomes "light ochraceous buff"[2] to "ochraceous buff"; on Sorbaud's potato dextrose agar the medium becomes "raw sienna" when viewed from below.

Pestalotia Leucothoës sp. nov.

Conidia 5-cellularia, constricta fusoideia, infera acuta 21.5–29.5 μ × 4.9–6.9 μ (plerumque 23.5–27.5 × 5.5–6.3 μ); superiores et mediae cellulae coloratae obscuriores quam inferior olivacea cellula. Superior cellula hyalina longa turbinata, setae 2–5 plerumque 3 vel 4 saepe erumpentes ex aliis punctis in corona cellulae superioris, raro ramosa similis cervi cornu, usque ad 39.5 μ longae, plerumque 20–33 μ. Conidiophoris plerumque 6–14 μ longis, usque ad 16 μ. Acervulis 75–150 μ in diam., subglobosis, plerumque hypophyllis.

Conidia 5-celled, narrow fusiform, acute at base, erect or usually curved, only slightly constricted at septae 21.5–29.5 μ long (usually 23.5–27.5 μ; ave. 25.9 μ) by 4.9–6.9 μ wide (usually 5.5–6.3 μ; ave. 6.2 μ); upper and middle colored cells darker than the lowest olivaceous cell, guttulate; colored cells 13.8–18.7 μ (usually 15.7–17.7 μ; ave. 16.0 μ). Apical hyaline cell long conic,

[2] Ridgeway's Color Standards.

setae 3 or 4, typically 3, rarely 2 or 5, often arising from different points on the crown of the apical cell; one or two setae sometimes arising below the summit, rarely branched like a staghorn, 3.9–39.5 μ long (usually 20–33 μ; ave. 26.1 μ); basal cell, long acute tapering to pedicel; pedicel 0–15.7 μ long (usually 5.9–13.8 μ; ave. 9.6 μ). Pustules 75–150 μ diam., subglobose, largely hypophyllous, the black contents issuing in coils surrounded by torn epidermis, or oozing forth as a globose head, staining the matrix.

On foliage of *Leucothoë Catesbaei* following infections of other fungi and winter injury (PLATE 32 *B* AND *C*). A secondary pathogene. Type locality, Springfield, N. J. August, 1931.

On potato dextrose and Sorbaud's potato dextrose agars, a heavy white aerial growth of mycelium is produced with abundant sporulation appearing after 6 days in tubes and 12 days on plates. Sporulation is sparce on lima bean and bean pod agars and the vegetative growth is largely embedded in the media. On corn meal and prune agar, growth is entirely submerged in the media and sporulation is either limited to the point of inoculation or absent entirely in petri dishes. When grown on potato dextrose agar, the medium becomes "pale yellow-orange"; on Sorbaud's potato dextrose "tawny olive."

Pestalotia Cibotii sp. nov.

Conidia 5 cellularia, anguste fusoideia, 19.7–29.5 × 4.9–6.8 μ (plerumque 21.5–27.5 × 4.9–5.9 μ). Mediae coloratae cellulae olivaceae, duae superiores paulo obscuriorés inferioribus. Superior cellula hyaline turbinata, globosa in apice, setae 3 vel 4, raro 2 vel 5, erumpentes ex aliis punctis in globosa apice superioris cellulae, 5.9–27.6 μ longae (plerumque 15.7–19.7 μ). Conidiophoris brevibus, 1.9–5.9 μ longis. Acervuli 120–175 μ in diam., plerumque epiphilli.

Conidia 5-celled, slender fusiform, usually erect or slightly curved, very slightly constricted at septae, 19.7 29.5 μ long (usually 21.5–27.5 μ; ave. 24.7 μ) by 4.9–6.8 μ wide (usually 4.9–5.9 μ; ave. 5.7 μ); median colored cells 13.8–17.7 μ (mostly 15.7–17.7 μ; ave. 16.0 μ), olivaceous, upper two only very slightly darker than the lower, sometimes umber. Apical hyaline cell, conic rounded at the summit; basal cell, broad acute. Setae 3 or 4, typically 4, rarely 2 and 5, arising from different points on the rounded top of the apical cell, 5.9–27.6 μ long (usually 15.7–19.7 μ; ave. 17.0 μ). Pedicel short, straight, 1.9–5.9 μ long (usually 1.9–4.9 μ; ave. 3.9 μ). Pustules largely epiphyllous,

the black contents issuing in coils or as cones, subglobose, 120–175 μ diameter.

Parasitic on living fronds of *Cibotium Schiedei* under greenhouse conditions (PLATE 32 *D*). Type locality, Rutherford, N. J. August 20, 1932.

In culture, thick closely woven mats of aerial mycelium are formed on potato dextrose and Sorbaud's potato dextrose agars which with age become tinged with "naples yellow." Sporulation is fairly abundant on both media; in plates the acervuli are all embedded in the medium, but in tubes spores are produced abundantly in wet black glistening drops. Sporulation also occurs abundantly in tubes of lima bean agar, and less abundantly in tubes of corn meal and prune agars. It does not sporulate in plates of bean pod agar.

Physiologically these organisms can be distinguished by the presence or absence of sporulation on various media. *P. Aucubae* sporulates abundantly on potato dextrose, lima bean and bean pod agars but sparsely on Sorbaud's potato dextrose agar. *P. Leucothoës* either fails to sporulate on lima bean and bean pod agars in plates or does so only at the point of inoculation, and sporulates abundantly on Sorbaud's potato dextrose agar. *P. Cibotii* fails to sporulate at all on plates of bean pod agar, and produces numerous embedded acervuli on plates of Sorbaud's potato dextrose agar and lima bean agar. Their habits of growth on lima bean agar are quite distinct (PLATE 32, *H*, *I*, *J*).

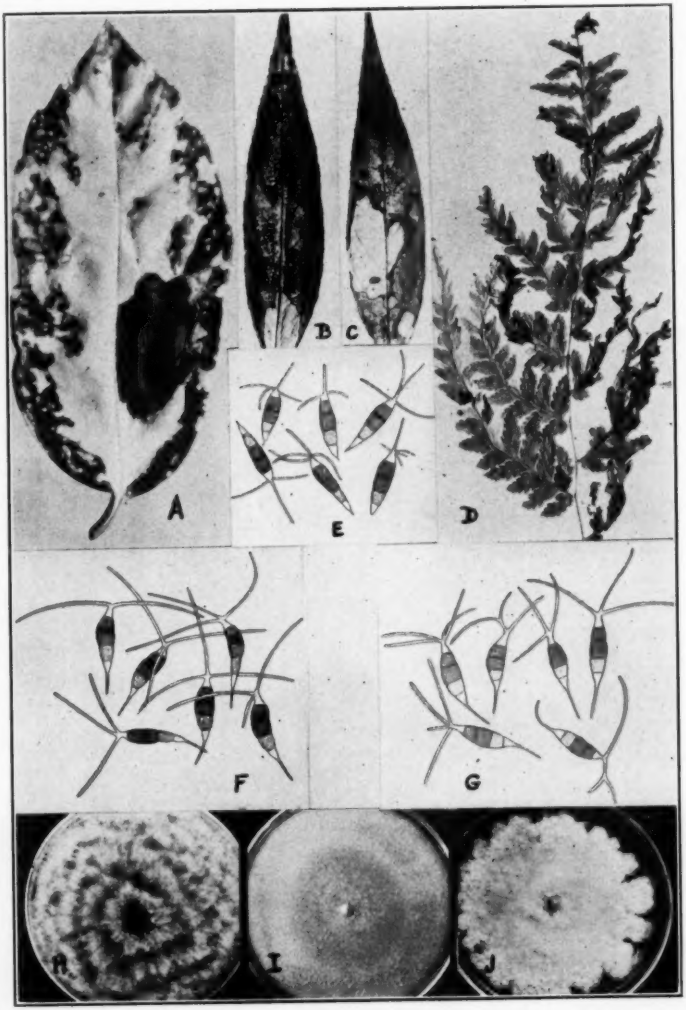
Morphologically *P. Aucubae* may be distinguished by its wider spores, and by its long, widely divergent setae, preponderantly 3 per spore; *P. Leucothoës* by its shorter setae often arising at different points on the crown of the apical cell, typically 3 per spore but with approximately 30 per cent of the spores with 4 setae; *P. Cibotii* by its short setae, typically 4 per spore but with approximately 40 per cent with 3 setae, often arising from different points on the apical cell, and its extremely short pedicel.

Type specimens of these fungi have been deposited in the herbarium of the Department of Plant Pathology, Cornell University, Ithaca, New York and in the cryptogamic herbarium at Harvard University, Cambridge, Massachusetts.

AGRIC. EXPER. STATION,
NEW BRUNSWICK, N. J.

EXPLANATION OF PLATE 32

A, leaf spot of *Aucuba japonica* var. *variegata* caused by *Colletotrichum Pollaccii* Magn. secondarily invaded by *Pestalotia Aucubae; B, C*, leaves of *Leucothoë Catesbaei* showing winter injury and secondarily invaded by *Pestalotia Leucothoës; D*, frond of *Cibotium Schiedei* infected with *Pestalotia Cibotii; E*, spores of *Pestalotia Cibotii* × 440; *F*, spores of *Pestalotia Aucubae* × 400; *G*, spores of *Pestalotia Leucothoës* × 400; *H, I, J*, cultures of *P. Aucubae, P. Leucothoës* and *P. Cibotii* respectively, on lima bean agar; 10 days old.



PESTALOTIA SP.

THE PERFECT STAGE OF CERCOSPORA RUBI

FREDERICK A. WOLF[1]

(WITH 8 TEXT FIGURES)

INTRODUCTION

The writer undertook a study of *Cercospora Rubi* Sacc., the cause of a common leafspot disease of *Rubus*, because so little is known regarding this pathogen. As a result of this investigation it was found that this conidial stage, present upon the leaves during summer, is succeeded by a perithecial stage which is initiated during autumn, but does not mature until early in the following spring. The following account of this study is herewith presented, therefore, as a contribution to our knowledge of the morphology and development of this leafspot fungus on *Rubus*.

Apparently both wild and cultivated species of *Rubus*, including blackberries, dewberries, and raspberries, are subject to attack by this imperfect fungus. Moreover, it is of common occurrence and widespread distribution within the eastern United States, and, in certain years, it occasions severe premature defoliation, especially in commercial plantings. Undoubtedly, a decrease in yield proportional to the degree that such defoliation interferes with the storage of food in the turions, results from this leafspot disease.

In North Carolina, the fungus has been collected on *Rubus*

[1] Contribution no. 136 from the Cryptogamic Laboratories of Harvard University. An investigation conducted while the writer held a Research Fellowship at Harvard University. Acknowledgment is made of the courtesies extended, for the loan of type specimens of species of *Sphaerella* on *Rubus*, by Dr. Gustav von Moesz, Director, Hungarian National Museum, Budapest, Hungary, by Dr. G. Samuelsson, Naturhistoriska Riksmusset, Stockholm, Sweden, and by Dr. J. A. Stevenson, Bureau of Plant Industry, Washington, D. C. The writer is grateful to Dr. Charles Chupp, Cornell University, Ithaca, N. Y., who confirmed the identification of the conidial stage as *Cercospora Rubi* Sacc. and gave his opinion on synonymy. Lastly, he is appreciative of the encouragement and assistance given by Messrs. W. H. Weston, Jr., and D. H. Linder.

alleghaneinsis Porter, *R. argutus* Link, *R. hispidus* L., *R. procumbens* Muhl., *R. procumbens* var. *roribaccus* Bailey, *R. trivialis* Michx., *R. idaeus* L., *R. strigosus* Michx., *R. occidentalis* L., and *R. thyrsoides* Wimm. Among other species known to be suscepts are *R. fruticosus* L. and *R. imperialis* Cham. It appears probable that an exhaustive examination of collections in herbaria, to include both those in this country and abroad, would reveal that *Cercospora Rubi* is pathogenic to essentially all of the numerous species of *Rubus*.

CONIDIAL STAGE

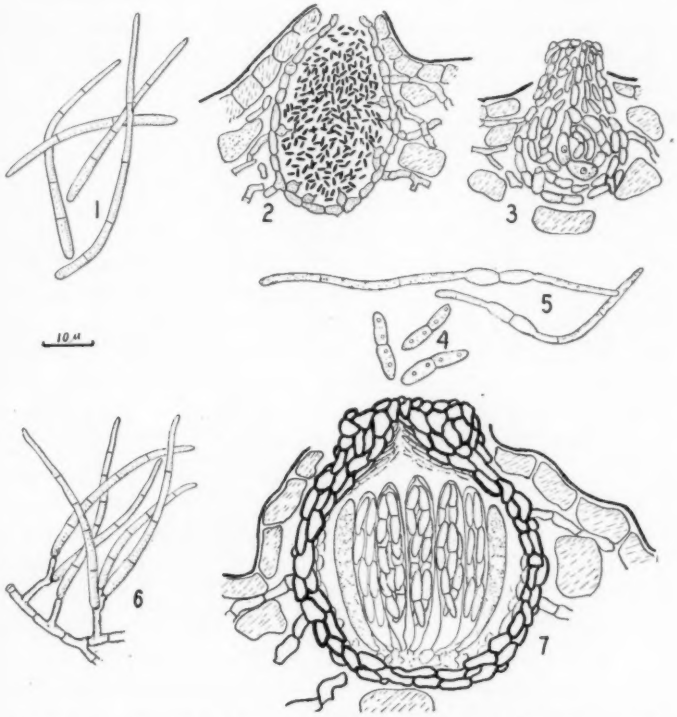
The conidial stage may be found at any time, throughout the entire summer since new lesions may appear at any time and since successive crops of conidia develop on old lesions. The lesions are characteristically large, brown, irregular leafspots (FIG. 8). They are most conspicuous on the upper leaf surface, because the covering of trichomes on the lower leaf surface renders the lesions less evident. They may, at first, be bordered with a margin that is darker brown or purplish, in which case the spots are definitely delimited, but as the surrounding uninvaded portions become pale green the margins pale out and the spots are thus less definitely delimited. The lesions may continue to enlarge until the leaves are shed by which time they may have fused and may have come to occupy most of the leaf surface. Finally the central portions of the lesions become grayish tan. They are early beset with numerous dark specks, the fascicles of conidiophores.

The conidiophores emerge from the stomates in short, small fascicles. They do not arise from a compact, extensive stroma, as occurs in many other species of *Cercospora*, although the invaded leaf tissues are occupied by an extensive, much-branched, intercellular mycelium. Conidia are formed and dislodged seriatim at the tips of the conidiophores, which become geniculate, as a result, and flexuose. The conidia (FIG. 1) are club-shaped, dilutely brown, curved, many-septate, and 40–130 × 3.0–4.5 μ. Their length is most commonly found to be 65–75 μ.

SPERMOGONIA AND PERITHECIAL INITIALS

The spermogonia and perithecial initials may be found to be present in the period extending from late August throughout

September and October. They occur on the lower surface of leaves that have recently been shed, and appear as numerous, black, pimple-like structures. Details of structure of these bodies



FIGS. 1–7. 1, conidia of *Cercospora Rubi* from lesions on leaves; 2, spermogonium in vertical section, showing pycnidial structure and spermatia; 3, stroma of perithecial initial in vertical section showing archicarp; 4, ascospores in *Mycosphaerella dubia;* 5, germinating ascospores; 6, conidia formed in culture from ascospores; 7, section of perithecium of *Mycosphaerella dubia*, embedded within the tissues of a decaying leaf of *Rubus*.

were studied by means of sections of lesions embedded in paraffin. To do this, leaf tissues were fixed, sectioned, and stained with Haidenhain's iron alum hematoxylin. An examination of these sections shows that the spermogonia (FIG. 2) are spherical to flask-shaped conceptacles, sunken within the leaf tissues and con-

taining myriads of minute, rod-shaped spermatia. By the time that the spermogonial aperture has developed, through which the spermatia are liberated, each spermogonium projects slightly above the leaf surface. The spermogonial wall is essentially a single cell-layer in thickness. The spermatia are budded off from ampulliform, spermatiferous cells that project from the inner surface of the spermogonium.

If the lower surface of lesions is examined with low magnification, it is impossible to distinguish between spermogonia and perithecial initials. In section, however, these latter structures are found to be loosely-aggregated stromata, flask-shaped in outline, whose cells tend to be arranged in series that look like deeply constricted hyphae. Within each stroma is a hyphal coil, the archicarp (FIG. 3), whose basal cells are larger and more deeply staining than the surrounding cells. The upper portion of this coil, the trichogyne, is more slender and extends to the surface of the stroma. No direct evidence has been found, in the case of this fungus, that the trichogynes function as receptive organs or that the spermatia constitute the male elements. In the light of our knowledge of certain other Ascomycetes, however, it may reasonably be assumed that fertilization must be accomplished by the fusion of the content of a spermatium with that of the ascogone. The perithecial initial is thereafter slowly transformed into the perithecium.

PERITHECIAL STAGE

The perithecial stage was found to develop when leaves bearing spermogonia and perithecial initials were permitted to remain out-of-doors throughout winter. By late April or early May, the perithecia were noted to be mature in these leaves. Perithecia as seen, with low magnification, are black points, sparsely scattered over the lower leaf surface. In section, the perithecia are observed to be immersed within the decaying leaves and to project to the surface by means of short papillae. They are minute, being approximately 40–60 μ in diameter (FIG. 7). Their wall is membranous, being constituted of a thin layer of brown, rather thick-walled cells. The asci are fasciculate, and paraphyses are lacking. The ascospores are distichate, hyaline, two-celled, and

11–14 μ long, the upper cell being slightly longer and wider than the lower (FIG. 4). The ascospores increase greatly in size in the brief period immediately prior to their discharge. Apparently their size can best be determined by measurement of freshly discharged ascospores. If microscopic slides are placed above moistened leaves, bearing perithecia, the ascospores will adhere to the slides.

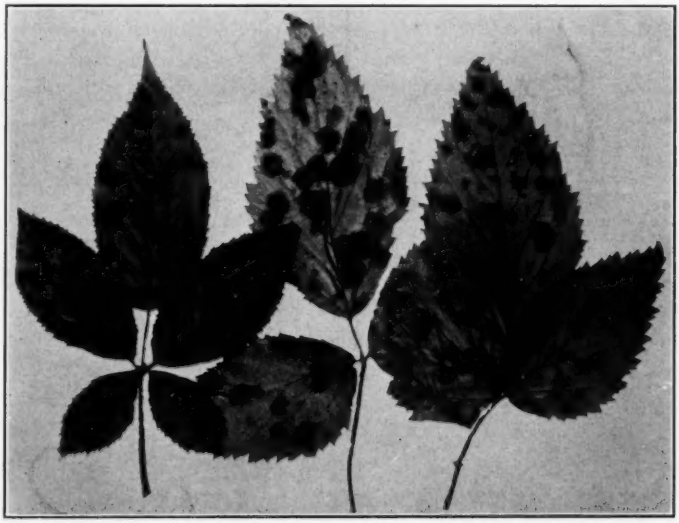


FIG. 8. Lesions on blackberry leaf (at left) and dewberry leaves (at right) produced by the conidial stage.

The morphological characteristics of the perithecial stage, just described, are clearly those of the genus *Mycosphaerella*. The genetic connection of the *Mycosphaerella* with the conidial stage was demonstrated by means of cultures. Isolations from ascospores were made by permitting the ascospores to be expelled onto the surface of corn meal agar plates. This was done by placing leaves, bearing perithecia, upon moistened blotters in the tops of Petri dishes. Agar films in the bottoms of the Petri dishes were then inverted over them. The ascospores, that were ejected, germinated by the emission of germ tubes, characteristically one from each extremity (FIG. 5). Within 72 hours, short

lateral branches, each bearing a single *Cercospora* conidium, had formed on the mycelium. These conidia were like those of *Cercospora Rubi* from leaf lesions. Within the next 2 or 3 days other conidia were abstricted from the same conidiophores, and they had become geniculate (FIG. 6). After the colonies had become more than a week old, conidia could no longer be found, and by this time the colonies had become compact, grayish mycelial mats.

Conidia taken from leaf lesions germinated on corn meal agar plates, and within a few days thereafter conidia were borne on the resulting mycelium. Furthermore the colonies that originated from conidia were entirely similar to those that arose from ascospores.

IDENTITY OF THE FUNGUS

The conidial stage of the fungus under consideration was first described as *Cercospora Rubi* by Saccardo (**4**), in 1876. His description was based upon collections on leaves of *Rubus fruticosus* from northern Italy and on leaves of *R. imperialis* from Argentina. In 1896, specimens of a fungus on *R. canadensis*, having smaller conidiophores and conidia, the conidiophores measuring 20–25 × 3 μ and the conidia 35–63 × 2–2.5 μ, were believed by Ellis and Everhart (**2**) to be distinct, and hence the fungus was named by them *Cercospora septorioides*. In 1917, a leafspot fungus on a species of blackberry from Texas, was described by Tharp (**6**) as *Cercospora Bliti*. An examination, by Chupp,[2] of type specimens of *Cercospora septorioides* and of *C. Bliti* shows that they are identical with *C. Rubi* and hence are synonymous.

The writer has not been able to compare *Cercospora Rubi* with *C. rubicola*, described by de Thümen (**1**), in 1881, as the cause of a leafspot of *Rubus fruticosus*, nor with *C. Garbiniana* described by Massalongo,[3] in 1902. Neither of these two species has been reported to occur within the United States. The lesions produced by *C. rubicola* are said to be large, ochraceous, and purple bordered, and therefore could be distinguished with difficulty, if at all, from those associated with *C. Rubi*. The conidia, how-

[2] Opinion communicated by letter.

[3] In Atti Mem. Acc. Agric., Sci. Lett, ed Arti. Verona. **3**: 153, *pl. 10, fig. 21*, 1902.

ever, are said to be of greater diameter than those of *C. Rubi*. *C. Garbiniana* is said to differ from *C. rubicola* mainly in having reddish conidiophores and shorter, narrower conidia; and from *C. Rubi*, in producing pale, reddish brown lesions, and in possessing cylindrical, less sturdy conidia. In the light of the writer's observations on the variation in character of the lesions produced by *C. Rubi*, and on the range in size of its conidia, that overlap those given for *C. rubicola* and *C. Garbiniana*, it appears probable that both of these are also synonymous with *C. Rubi*. This remains to be established, however, by comparative studies of the types, supplemented, perhaps, by studies of their development.

Another species of *Cercospora* on *Rubus* was recently described by Sydow.[4] It was collected, on *Rubus rosaefolius* Smith, in the Transvaal, and is characterized by its failure to produce definite leaf spots. Instead, an effuse, olivaceous growth of conidiophores and conidia is developed on the lower leaf surface, and this organism is unquestionably distinct from *C. Rubi*.

A survey of mycological literature dealing with *Mycosphaerella* (*Sphaerella*) on *Rubus* reveals the fact that 11 species of *Sphaerella* have been recorded to occur on species of *Rubus*. These are *Sphaerella Chamaemori* Karst., *S. fructicum* Starb., *S. idaeina* Hazsl., *S. innumerella* Karst., *S. Ligea* Sacc., *S. maculiformis* (Pers.) Auserw., *S. minoensis* Syd., *S. Rubi* Roark, *S. rubicola* McAlp., *S. rubina* Peck, and *S. Winteri* (Pass.) Sacc. An attempt was made to identify the fungus under consideration by comparison with the original descriptions of the above-named species, and by comparison with type specimens, and authoritatively determined specimens, in so far as these were available in the Farlow Herbarium or could be obtained through loans. An examination of type specimens of *Sphaerella idaeina* shows that the ascospores instead of being 14–16 μ long, as described by Hazslinsky (**3**), are found to range up to 26 μ. The type specimens of *S. fructicum* possess asci and ascospores of approximately the same size as the perithecial stage of *Cercospora Rubi*, but it is caulicolous. Its perithecia are of different distribution, size, and shape from the fungus under consideration, and its morphologic features are as described by Starbäck (**5**).

[4] In Ann. Myc. **22**: 433–434. 1924.

The type specimens of *S. Ligea* show that it develops in irregular, brown lesions on green leaves. Moreover, *S. Ligea* is not the perfect stage of *Septoria Rubi* Westd., as suggested by Saccardo. The writer is familiar with *Septoria Rubi* and with its perfect stage, *Mycosphaerella Rubi* Roark, both of which stages he has collected and observed.

Measurements of *S. Winteri* are not given in Saccardo's Sylloge Fungorum but an examination of authoritatively determined specimens from Hungary shows that its perithecia arise within large, concentrically zonate spots on green leaves and that it is considerably larger than the perithecial stage of *Cercospora Rubi*.

The ascospores of *Sphaerella Chamaemori*, *S. innumerella*, *S. minoensis*, or *S. rubicola* are approximately twice as long as those of the fungus with which the writer is dealing. *Sphaerella maculiformis* occurs on the decaying leaves of a variety of woody plants including oaks, chestnut, sycamore, and basswood. Although its ascospores correspond closely in size with the fungus under consideration, its perithecia and asci are much larger. There does not appear to be any likelihood that it is identical with the fungus whose conidial stage is *Cercospora Rubi* since these trees are not attacked by a fungus like *Cercospora Rubi*. The perithecia of *S. rubina* are approximately four times as large, the asci twice as large, and the ascospores twice as wide as those of the fungus under consideration.

It would appear unlikely that the perfect stage of a leafspot fungus that is as commonly prevalent and as widely distributed as this one on *Rubus* could have escaped being collected and described previously. It would also appear equally improbable that 11 species of *Sphaerella* could occur on *Rubus*. Certain of them appear to the writer to be synonymous, but he hesitates to assign any of them to synonomy without first having made a detailed study of their morphology and cycle of development. Neither has he been able to establish beyond a reasonable doubt that the organism under consideration is or is not one of the 11 so-called species of *Sphaerella* on *Rubus*. The problem of identity and synonomy of species of *Sphaerella* on *Rubus* must remain for some future investigation. It is believed, however, that least confusion would result, at this time, because the fungus under

consideration is so widely known in its conidial stage, and its identity could thus readily be established, if a new specific name were erected. It is proposed therefore to assign a new name to the fungus whose conidial stage is *Cercospora Rubi*, and it is briefly described as follows:

Mycosphaerella dubia sp. nov.

Syn. *Cercospora Rubi* Sacc. Nuovo Giorn. Bot. Ital. **8**: 188. 1876.

Cercospora septorioides Ellis & Ev. Publ. Field Mus. Bot. **1**: 94. 1896.

Cercospora Bliti Tharp, Jour. Myc. **9**: 108. 1917.

Peritheciis sparsis, hypophyllis, sphaeroideis, ostiolo papillaeformi praeditis, semi-immersis deinde erumpentibus, nigris, 40–60 μ diam.; ascis fasciculatis, clavatis, brevistipitatis, aparaphysatis, 8-sporis, apice tunica incrassatis; sporidiis bi-seriatis, hyalinis vel subhyalis, curvatis, oblongo-cylindricis, bilocularibus, loculis inaequalibus, quorum alter vel angustior vel latior, alter est longior, 11–14 μ longis, crassites sporidiorum est variabilis.

Spermogoniis autumno efformantis, hypophyllis, innato erumpentibus, punctiformibus, sparsis, nigris, globosis, 20–25 μ diam.; spermatiis bacillaribus, hyalinis, 2–3 $\times$ 1–1.5 μ.

Hab. in foliis dejectis *Rubi* sps.

Status conidicus: Maculis aridis, brunneis, deinde griseolis, magnis, subcircularibus vel irregularibus, saepe confluentibus; hyphis epiphyllis, fuscis, stomatibus pertussis, sursum geniculato-flexuosis; conidiis longe clavulatis, sursum attenuatis, curvulis 40–130 $\times$ 3–4.5 μ, pluriseptatis, subhyalis vel dilute fuscis.

Hab. ad folia viva *Rubi* sps., frequens.

For the convenience of mycologists type specimens have been deposited in the Farlow Herbarium, Harvard University, Cambridge, Mass. Co-type specimens have been deposited in the Herbarium of Mycological Collections, U. S. Dept. of Agriculture, Washington, D. C., the Hungarian National Museum, Budapest, Hungary, and the Naturhistoriska Riksmusset, Stockholm, Sweden.

SUMMARY

This investigation deals with the morphology and development of a common leafspot fungus of many species of *Rubus* that is known in its conidial stage as *Cercospora Rubi* Sacc.

This fungus has been found to possess a perithecial stage that is initiated in autumn by the formation of spermogonia and archicarps. The perithecia mature in spring.

Genetic connection of the conidial and perithecial stages has been established by growth in culture.

The conidial stage is identical with *Cercospora septorioides* Ellis & Ev. and *C. Bliti* Tharp., both of which names are assigned to synonomy. *C. rubicola* and *C. Garbiniana* may also be synonymous.

The perithecial stage cannot be identified with previously described species of *Sphaerella* on *Rubus* and is herein briefly described as *Mycosphaerella dubia*.

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LITERATURE CITED

1. **de Thümen, F.** Contributiones ad floram mycologicam lusitanicam III. Instituto **28**: [1–54]. 1881. (page 12.)
2. **Ellis, J. B., & Everhart, B. M.** *Cercospora septorioides*. Publ. Field Mus. Bot. **1**: 94. 1896.
3. **Hazslinszky, F.** Magyarország ès társországainak sphaeriái. Math. Term. Közlem. **25**[2]: 65–397. illus. 1892. (page 104, *pl. V, fig.* 21.)
4. **Saccardo, P. A.** Fungi Veneti novi vel critici. Nuova Giorn. Bot. Ital. **8**: 161–211. 1876. (page 188.)
5. **Starbäck, K.** Anteckningar öfver några Skandinaviska Pyrenomyceter. Bih. Sv. Vet.-Akad. Handl. **14(3)**[5]: 1–18. 1889. (page 17, *pl.* 1, *fig.* 5*a*, *b*, *c*.)
6. **Tharp, B. C.** Texas parasitic fungi. Mycologia **9**: 105–124. 1917. (page 108.)

THE HYDNACEAE OF IOWA IV. THE GENERA STECCHERINUM, AURISCALPIUM, HERICIUM, DENTINUM AND CALODON

L. W. MILLER

(WITH PLATE 33)

The pileate members of the Hydnaceae of North America have been carefully studied and treated by Dr. Howard J. Banker. His paper, "A contribution to a revision of the North American Hydnaceae" (1906), and the type studies which appeared in a number of papers from 1912 to 1914 are particularly valuable. Additional knowledge has been contributed by Coker, Lloyd, Beardslee and others. With a few exceptions Banker's conception of the genera and species is followed, and only such changes are made as seem to the writer desirable or in accord with more recent rules of botanical nomenclature.

STECCHERINUM S. F. Gray, Nat. Arr. Brit. Pl. 1: 651. 1821

Pileus reflexed to laterally substipitate, or entirely resupinate, tough or occasionally subfleshy; spores minute, smooth, ovoid to oblong, hyaline. Growing on wood.

KEY TO THE SPECIES OF STECCHERINUM

1. Mostly resupinate, sometimes reflexed or rarely distinctly pileate; with numerous, incrusted, elongated, cylindrical or clavate and emerging cystidia ..... (2)
1. Distinctly pileate, rarely reflexed; cystidia, if present, short, fusiform and relatively few in number ..... (4)
   2. Resupinate, sodden, corky-fibrous; spines often crowded and coalesced at the base; spores 6–7 × 3–4 μ ..... 5. *S. setulosum.*
   2. Resupinate, reflexed or rarely entirely pileate, dry, soft, leathery; spines generally free; spores 3–5.5 × 2–3.5 μ ..... (3)
3. Margin entire, soft; spines terete or angular, more or less uniform in shape and size; seldom darker than vinaceous cinnamon; spores 3–4 × 2–2.5 μ ..... 1. *S. ochraceum.*
3. Margin radially and irregularly fimbriate; spines terete or greatly flattened, often serrate, irregular in size; more orange than vinaceous cinnamon; spores 4–5.5 × 3–3.5 μ ..... 2. *S. lacticolor.*
   4. Spines 6–18 mm. in length; cystidia short, fusiform; spores 4–6 × 2.5–3.5 μ ..... 3. *S. septentrionale.*

4. Spines 1–5 mm. in length; cystidia fusiform or absent; spores 5 μ or less in length ..... (5)
5. Texture soft, fibrous-fleshy; cystidia absent; hyphae with 2 prominent clamp connections at many septa; spores 4–5 $\times$ 2–2.5 μ. 4. *S. pulcherrimum.*
5. Texture leathery, fibrous; cystidia present or absent; clamp connections single or absent; spores 4 μ or less in length ..... (6)
6. Faintly zoned near the margin only; spores short cylindrical, 2.5–3 $\times$ 1–1.25 μ ..... 6. *S. adustum.*
6. Distinctly zonate; spores ovoid to ellipsoid ..... (7)
7. Cystidia fusiform; spores ellipsoid, 3–4 $\times$ 2 μ ..... 7. *S. rawakense.*
7. Cystidia absent; spores ovoid, 2–2.5 $\times$ 1.5 μ ..... 8. *S. pusillum.*

1. STECCHERINUM OCHRACEUM (Fries) S. F. Gray, Nat. Arr. Brit. Pl. **1**: 651. 1821. (FIG. 1)

Hydnum ochraceum Pers. ex Fries, Syst. Myc. **1**: 414. 1821.

Hydnum Rhois Schw. Schr. Nat. Gaz. Leipzig **1**: 103. 1822.

Hydnum pudorinum Fries, Elench. **1**: 133. 1828.

Hydnum flabelliforme Berk. Lond. Jour. Bot. **4**: 306. 1845.

Hydnum plumarium Berk. & Curt. Grevillea **1**: 97. 1873.

Climacodon ochraceus (Fries) Karst. Bidr. Finl. Nat. Folk. **37**: 98. 1882.

Climacodon pudorinus (Fries) Karst. Bidr. Finl. Nat. Folk. **37**: 97. 1882.

Leptodon pudorinum Quél. Fl. Myc. Fr. 440. 1888.

Hydnum conchiforme Sacc. Syll. Fung. **6**: 458. 1888.

Mycoleptodon ochraceum Pat. Tax. Hymén. 116. 1900.

Steccherinum Rhois (Schw.) Banker, Mem. Torrey Club **12**: 126. 1906.

Hydnum alnicolum Vel. České houby. 745. 1922.

Fructification resupinate, reflexed or entirely pileate and laterally sessile, fibrous, tough, slightly separable, the upper surface of the pileus soft, tomentose, sulcate zoned, cartridge buff; hymenial surface pinkish cinnamon to vinaceous cinnamon; margin whitish, pubescent, subfimbriate, usually wider in the resupinate portions than in the reflexed; spines 2 mm. or less in length, crowded, 4–6 per mm., slender, subulate, pointed, terete or flattened, often divided, gradually decreasing in length toward the margin; hyphae thick-walled, 2–6 μ in diameter, coarser and less compactly interwoven next to the substratum or on the upper surface of the pileus, with scattered clamp connections, thin-walled in the sub-hymenial region and not exceeding 3.5 μ in diameter; cystidia 20–100 $\times$ 4–9 μ, numerous, projecting singly

from the sides and the apex of the spines, elongated, clavate to cylindrical, slightly curved, arising from the parallel hyphae in the axial portion of the spine, thick-walled, usually heavily incrusted; basidia 12–15 × 3–5 μ, clavate; spores 3–4 × 2–2.5 μ, obovate, smooth, hyaline.

This species is recognized by its tough texture, its ochraceous color, the numerous elongated and projecting cystidia and the small, obovate spores. It varies much in habit of growth. *Hydnum flabelliforme* Berk. and *Hydnum Rhois* Schw. seem to refer to the more pileate variations of the species.

Abundant in Iowa on dead wood of deciduous trees; collected in all seasons. Apparently common throughout the United States.

2. STECCHERINUM LAETICOLOR (Berk. & Curt.) Banker, Mycologia **4**: 316. 1912

Hydnum laeticolor Berk. & Curt. Grevillea **1**: 99. 1873.

Irpex laeticolor (Berk. & Curt.) Morgan, Jour. Cinc. Soc. Nat. Hist. **10**: 15. 1887.

Mycoleptodon laeticolor (Berk. & Curt.) Pat. Tax. Hymén. 116. 1900.

Resupinate or with a slightly detached or reflexed margin, soft spongy and slightly tough in texture, somewhat separable, zinc orange to orange-cinnamon; margin floccose, radially fimbriate, white or lighter in color; spines 2 mm. or less in length, irregular, flattened, serrate or divided, Irpex-like, obtuse; hyphae 3–6 μ in diameter, thick-walled, with scattered clamp connections; cystidia 5–9 μ in diameter, cylindrical, elongated, incrusted, projecting at the sides and apex of the spines; basidia 8–15 × 3–5 μ, clavate; spores 4–5.5 × 3–3.5 μ, ellipsoid, smooth, hyaline.

S. laeticolor closely resembles *S. ochraceum*. It may be separated in most cases by the more reddish color, the soft texture, the radially fimbriate margin, the irregular teeth and the larger spores. The larger spores are diagnostic. A fragment of the type at the New York Botanical Garden has been examined.

Uncommon in Iowa; collected in August and February. Widely reported from the central and eastern United States. Apparently more common in the southern states.

3. STECCHERINUM SEPTENTRIONALE (Fries) Banker, Mem. Torrey Club **12**: 130. 1906. (FIG. 3)

Hydnum septentrionale Fries, Syst. Myc. **1**: 414. 1821.
Climacodon septentrionalis Karst. Rev. Myc. **3**: 20. 1881.
Creolophus septentrionalis (Fries) Banker, Mycologia **5**: 293. 1913.

Fructification tough, fibrous, generally consisting of many sessile, horizontal pilei which are imbricate, confluent at the base and arising from a small, single point of attachment, reported to weigh as much as 60 lbs. (Lloyd); single pilei more or less flattened, usually 3–15 cm. wide, 2–15 cm. long, reported up to 30 cm. wide and 20 cm. long, fibrous and pubescent on the upper surface, not zoned, reported white, becoming coarsely wrinkled and ochraceous buff in the herbarium; margin abrupt, slightly incurved; spines 6–18 mm. in length, 2–3 per mm., slender, terete, pointed, entire, reported milk-white, becoming prussian red to pecan brown in the herbarium; hyphae 3–6 μ in diameter, compactly interwoven, often agglutinated, with few clamp connections, usually accompanied by larger, scattered and apparently gelatinized hyphae measuring 6–11 μ in diameter; cystidia 30–50 $\times$ 10–16 μ, short, fusiform, thick-walled, projecting 10–25 μ; basidia 12–18 $\times$ 4–5 μ, clavate; spores 4–6 $\times$ 2.5–3.5 μ, ellipsoid, smooth, hyaline.

S. septentrionale is similar to *S. pulcherrimum* and the two have often been confused. The former species, however, is dryer, tougher and remains a paler color in the herbarium. This species may be distinguished also microscopically by the presence of the fusiform cystidia and the absence of the two clamp connections at the septa of hyphae. The many horizontal and usually joined pilei are very characteristic for the species.

Unfortunately I have been unable to see a living specimen of *S. septentrionale*. The characters noted above for fresh fructifications have been taken largely from the description of Banker. I find a considerably greater range in the length of the spine. Several specimens quite obviously correctly determined have spines not exceeding 6 mm. in length. Banker reports the spines as 17–18 mm. in length.

Collected on maple in August; not common in Iowa. Apparently occurs throughout the central and the eastern United States.

4. STECCHERINUM PULCHERRIMUM (Berk. & Curt.) Banker, Mem. Torrey Club **12**: 129. 1906. (FIG. 7)

Hydnum pulcherrimum Berk. & Curt. Hooker's Jour. Bot. and Kew Gard. Misc. **1**: 235. 1849.

Hydnum friabile Fries, Nova Acta Soc. Sci. Upsal. III. **1**: 106. 1851.

Steccherinum agaricoides (Swartz) Banker, Mem. Torrey Club **12**: 130. 1906.

Creolophus pulcherrimus (Berk. & Curt.) Banker, Mycologia **5**: 294. 1913.

Creolophus agaricoides (Swartz) Banker, Mycologia **5**: 294. 1913.

Fructification pileate, sessile, solitary to imbricate, dimidiate to flabelliform, often arising from a resupinate base, sometimes bell-shaped or convex, variable in size, soft, pliable, somewhat tough, fibrous, juicy when fresh and sometimes containing a milky white, sticky sap, soft and gummy to brittle when dry, whitish at first becoming light buff, tawny to liver brown in the herbarium; individual pilei up to 15 cm. in width and 8 cm. in length, 0.2–10 mm. thick at the base, densely fibrous or hairy on the upper surface; margin very thin; spines 1–6 mm. in length, usually about 5 mm., 4–5 per mm., slender, terete, pointed, entire, tawny in herbarium; hyphae 4–6 μ in diameter, loosely packed in the pileus, with slightly thickened walls, with numerous clamp connections, often two at each septum, more fragile and with fewer clamp connections in the spines, 2–3 μ in diameter; basidia 12–18 $\times$ 3–4 μ, clavate; spores 4–5 $\times$ 2–2.5 μ, variable, ellipsoid, slightly depressed laterally, smooth, hyaline.

The soft, somewhat fibrous texture, the tawny color, the white, sticky sap when fresh and the two clamp connections at many of the septa of the coarse hyphae are well marked characters in this species. These double clamp connections are sometimes few in number but I have never failed to find them in the several dozen specimens examined. I have examined, at The New York Botanical Garden, the *Murrill* and *Harris* specimen, No. *1095*, which Banker regards as representing *Hydnum agaricoides* Swartz. It seems to be a young growth of *S. pulcherrimum* and agrees in every microscopic detail with a typical fructification. It varies only in being slightly more fleshy and has short undeveloped teeth.

Rather uncommon in Iowa, occurring on dead wood of various

frondose species from August to September. The seasonal range is probably greater than reported. Only two specimens in our herbarium have the date given. Widely reported from the eastern half of the United States.

5. **Steccherinum setulosum** (Berk. & Curt.) comb. nov. (FIG. 2)
Hydnum setulosum Berk. & Curt. Grevillea 1: 100. 1873.

Resupinate, effused, adnate, thick, soft corky-fibrous, waterlogged when fresh, avellaneous, contracting and curling upon drying but not cracking, cinnamon to army brown in the herbarium; margin concolor; spines 2–9 mm. in length; variable, usually crowded and coalesced more or less at the base, terete, cylindrical and obtuse to subulate and acute, sometimes flattened and branched; hyphae 1.5–4 μ in diameter, thin-walled, with scattered clamp connections, others thick-walled, compactly arranged in older fructifications; cystidia 30–90 $\times$ 4–10 μ, numerous, long, cylindrical to clavate, obtuse, incrusted, arising from the thick-walled hyphae in the axial portion of the spine and in the subiculum, projecting; basidia 20–25 $\times$ 6–7 μ, clavate, with 4 sterigmata; spores 6–7 $\times$ 3–4 μ, ellipsoid, smooth, granular or guttulate.

S. setulosum is characterized by the sodden, soft fibrous texture, the rather long, more or less coalesecd spines, the numerous, elongated cystidia and the large spores. This species received its name because of the numerous cystidia projecting uniformly from the sides of the spines, a character readily detected under a lens.

A minute fragment of the type of *Hydnum setulosum* was examined at The New York Botanical Garden. It is characterized by smaller and less crowded spines than in Iowa specimens and according to the original description differs also in the possession of a broad sterile border. Iowa specimens were identical in color, texture and microscopic structure. An Iowa specimen was sent to Kew in order that it might be compared with a larger portion of the original specimen. Miss Wakefield reports that it agrees with the type.

Collected in September and October on dead wood and an old sporophore of *Fomes*. Relatively uncommon in Iowa. Apparently reported only from the type locality in Alabama.

6. STECCHERINUM ADUSTUM (Schw.) Banker, Mem. Torrey Club **12**: 132. 1906. (FIG. 4)

Hydnum adustum Schw. Schr. Nat. Ges. Leipzig **1**: 103. 1822.

Pileus tough, fibrous, brittle in the herbarium, dimidiate, flabelliform or reniform, with a resupinate base, laterally sessile or more often with a short lateral stipe, occasionally with an eccentric or central stipe, often subimbricate with abortive or fully formed pilei arising from the upper surface of lower ones, 3–7 cm. wide, 2–4 cm. long, more or less flattened, depressed toward the substratum or stipe, with a finely pubescent, slightly duller than cinnamon buff surface, faintly zoned toward the margin; spines 1–3 mm. in length, 4–5 per mm., slender, terete, angular or flattened, sometimes coalescing, often divided at the apex, whitish at first, becoming russet in the herbarium; hyphae 2–5 μ in diameter, quite homogeneous in the pileus, thick-walled in the pileus and axial portion of the spines, with scattered clamp connections; basidia 8–15 $\times$ 3 μ, clavate; spores 2.5–3 $\times$ 1–1.25 μ, minute, short cylindrical, smooth, hyaline or granular.

This species may be recognized by the more or less dry, tough, reniform pileus which usually has a short, stout, lateral stipe and by the minute spores. *Steccherinum pusillum* (Brot.) Banker is similar but may be separated by the distinctly zonate pileus and the ovoid spores.

Collected in Iowa on dead wood from July to November. Not common. Apparently widely distributed in the eastern United States and Canada.

7. STECCHERINUM RAWAKENSE (Pers.) Banker, Mycologia **4**: 312. 1912. (FIG. 5)

Hydnum rawakense Pers. Freyc. Voy. Aut. Monf. Bot. 175. 1826.
Hydnum reniforme Berk. & Curt. Jour. Linn. Soc. **10**: 325. 1869.
Hydnum glabrescens Berk. & Rav. Grevillea **1**: 97. 1873.
Hydnum guaraniticum Speg. Anal. Soc. Cl. Argent. **17**: 74. 1884.
Hydnum basiasperatum P. Henn. Hedwigia **36**: 199. 1897.
Steccherinum Morgani Banker, Mem. Torrey Club **12**: 127. 1906.
Steccherinum reniforme (Berk. & Curt.) Banker, Mem. Torrey Club **12**: 127. 1906.

Pileus tough, fibrous, brittle in the herbarium, horizontal, reniform to flabelliform, more or less flattened, laterally sessile or substipitate, occasionally with a small, resupinate base, closely

imbricate, often confluent toward the base; upper surface sulcate-zonate, glabrous or subpubescent with very short, coarse hairs, cinnamon in the herbarium; spines 2.5 mm. or less in length, 4–5 per mm., slender, terete or angular, subulate, pointed, chestnut-brown in the herbarium; hyphae 2.5–6 μ in diameter, thick-walled in the pileus and the axial portion of the spines, without clamp connections; cystidia 15–30 $\times$ 5–9 μ, short fusiform, pointed thick-walled, entirely hymenial, projecting about 10 μ; basidia 12–15 $\times$ 4 μ, clavate; spores 3–4 $\times$ 2 μ, ellipsoid, smooth, hyaline.

This species resembles *Steccherinum adustum* in size, texture and general appearance but the pileus is usually less stipitate and more distinctly zoned, the spores are larger and cystidia are present. The cystidia and larger spores distinguish this species also from *Steccherinum pusillum* (Brot.) Banker. I have examined the specimen at The New York Botanical Garden referred by Morgan to *Hydnum glabrescens* Berk. & Rav. and upon which Banker erected *Steccherinum Morgani*. It is identical in microscopic detail with *Steccherinum rawakense* (Pers.) as that species is understood by Banker. The slight differences in the external characters of this specimen from the typical condition in *S. rawakense* do not seem sufficient grounds for separating it as a distinct species. The type of *Hydnum glabrescens* was also studied at The New York Botanical Garden. The original description of *Hydnum rawakense* Pers. has not been seen. The citation is taken from Banker.

The single specimen in the University of Iowa herbarium was collected in Johnson County, Iowa, in November. Rare. Reported from several scattered localities in the eastern United States.

8. STECCHERINUM PUSILLUM Brot. ex Banker, Mycologia **4**: 313. 1912. (FIG. 6)

Steccherinum adustulum Banker, Mem. Torrey Club **12**: 133. 1906.

Pileus tough, fibrous, brittle in the herbarium, laterally stipitate or sessile, reniform or somewhat irregular, whitish with slightly darker zones on the upper surface; spines 2 mm. or less in length, 5–7 per mm., slender, flexuous, terete to flattened; cystidia absent; hyphae 2.5–4 μ in diameter, thick-walled, with-

out clamp connections; basidia 10–15 × 3.5–5 μ, clavate; spores 2–2.5 × 1.5 μ, ovoid, smooth, hyaline.

S. pusillum is reported from Iowa but no specimen believed to represent this species was found in the University of Iowa herbarium. The above notes were taken from a study of the type of *S. adustulum* at The New York Botanical Garden. This species seems closely related to *S. rawakense* and *S. adustum*.

AURISCALPIUM S. F. Gray, Nat. Arr. Brit. Pl. 1: 650. 1821

Pileus entire or lobed, laterally stipitate, leathery; spines slender, subulate, spores hyaline, small. Growing on cones of conifers.

AURISCALPIUM VULGARE S. F. Gray, Nat. Arr. Brit. Pl. 1: 650. 1821. (FIG. 10)

Hydnum Auriscalpium L. ex Fries, Syst. Myc. 1: 406. 1821.

Pleurodon Auriscalpium (Fries) Quél. in Cooke & Quélet, Clav. Hymen. 198. 1878.

Auriscalpium vulgare Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

Leptodon Auriscalpium Quél. Ench. Fung. 192. 1886.

Auriscalpium Auriscalpium (Fries) Banker, Mem. Torrey Club 12: 178. 1906.

Pileus 1–2 cm. wide, horizontal, cordate to reniform, supported laterally, slightly convex, leathery, finely hispid, bister to almost black; stipe vertical, 1–6 cm. long, slender, leathery, finely hispid, bister to almost black in upper portion, swollen, spongy and a lighter shade of brown at the base; spines 1.5 mm. or less in length, 4–5 per mm., slender, terete, acute; hyphae 2–6 μ. with thickened walls, few clamp connections, faintly colored, compactly arranged, fascicled to some extent in the pileus and the stipe; cystidia occasionally present, 15–30 × 3–6 μ; thin-walled, subulate; basidia 15–20 × 5–6 μ, clavate, with 2–4 sterigmata; spores 4.5–5 × 3.5–4 μ, obovate, apiculate or slightly attenuated at the base, smooth or minutely papillose, hyaline.

This species is recognized by the slender, vertical stipe, the reniform, laterally supported pileus, the dark color and hispid character of the stipe and pileus.

On decaying cones of coniferous species; June to November. Rare in Iowa. Reported from Oregon, Arizona and from various localities in central and eastern United States.

HERICIUM Pers. ex S. F. Gray, Nat. Arr. Brit. Pl. 1: 652. 1821

Fleshy or subfleshy, pulvinate or branched; spines long, pendent; gloeocystidia usually present; spores subspherical, guttulate. Growing on wood.

KEY TO THE SPECIES OF HERICIUM

1. Fructification consisting of a mass of branching processes; spores 4–7 μ in diameter.....(2)
1. Fructification massive, rarely showing a tendency to form branching processes; spines long, more or less uniform in size and distribution, 1–4 cm. in length.....3. *H. Erinaceus.*
   2. Intricately branched and anastomosing; spines seldom over 6 mm. in length, more or less uniformly distributed on the underside of the slender branches; spores 4–5 × 3–4 μ in diameter..1. *H. laciniatum.*
   2. Main branches few, short, relatively stout; spines 5–15 mm. in length, typically in fascicles at the ends of the terminal branches or on short lateral spurs; spores 5.5–7 μ in diameter.....2. *H. coralloides.*

1. HERICIUM LACINIATUM Leers ex Banker, Mem. Torrey Club 12: 114. 1906. (FIG. 8)

Hydnum coralloides Scop. ex Fries, Syst. Myc. 1: 408, in part. 1821.

Medusina coralloides Chev. Fl. Gen. Env. Paris 1: 279. 1826.

Manina flagellum Scop. ex Banker, Mycologia 4: 276. 1912.

Fructification consisting of a repeatedly dividing and more or less anastomosing mass of branches, arising from a common base which usually penetrates the substratum, soft, fleshy, whitish. becoming cream-buff to russet in the herbarium; spines 1–6 mm, in length, terete, subulate, acute, more or less uniformly distributed on the under side of the branches, often borne on the upper side of the terminal branchlets; hyphae 3–20 μ in diameter, with occasional clamp connections; gloeocystidia 5–7 μ in diameter, arising from the subhymenial region and penetrating the hymenium, containing a yellowish, refractive material; basidia 20–35 × 5–6 μ, clavate, slightly guttulate; spores 4–5 × 3–4 μ, smooth, hyaline, 1-guttulate.

This species is recognized by the characteristic fructification. It is more intricately branched than *H. coralloides*, has shorter and more uniformly distributed spines and smaller spores. According to Banker (1906 & 1912) this species was named *Hydnum flagellum* by Scopoli (1772), *Hydnum laciniatum* by Leers (1775), and *Hydnum ramosum* by Bulliard (1791) and is distinct from *H. coralloides* Scop. The pre-Friesian synonymy has not been

checked. Fries in the Systema Mycologicum considered Leers and Bulliard's names as synonyms of *Hydnum coralloides* Scop., the viewpoint followed by most mycologists since the time of Fries. I have examined a considerable number of specimens of *coralloides* Scop. from Europe and America and it seems apparent, as Banker states, that the forms commonly included under this name represent two valid species.

Common in Iowa on dead logs of frondose species from August to November. Widely reported from the United States. Probably more generally known as *Hydnum coralloides*.

2. HERICIUM CORALLOIDES Scop. ex S. F. Gray, Nat. Arr. Brit. Pl. 1: 652. 1821. (FIG. 14)

Hydnum coralloides Scop. ex Fries, Syst. Myc. 1: 408, in part. 1821.

Dryodon coralloides (Fries) Quél. in Cooke & Quél. Clav. Hymen. 198. 1878.

Friesites coralloides (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

Fructification usually consisting of several relatively short, stout, main branches from which short and more slender terminal branches arise, soft, fleshy, whitish, becoming warm-buff to various shades of brown in the herbarium; spines 5–15 mm. in length, terete, acute, usually arising in terminal clusters or on short lateral nodules from the sides of the main branches; hyphae 3–20 μ in diameter, with occasional clamp connections; gloeocystidia 6–8 μ in diameter, sometimes emerging up to 25 μ; basidia 20–45 $\times$ 5–6 μ, clavate; spores 5.5–7 μ, spherical, smooth, hyaline, 1-guttulate.

Hericium coralloides merges gradually into the form commonly known as *H. Caput-ursi*. Whether the two should be regarded as distinct is uncertain. I have examined a great many specimens of both species at The New York Botanical Garden and in other herbaria and am unable to distinguish the two by microscopic characters. A gradual series may be built up from one to the other. The two extremes of the series are quite different in general appearance. The branching fructification of *Hericium coralloides* resembles closely *H. laciniatum* but may be separated at once by the larger spores. The two have often been confused. Out of ten specimens of *H. coralloides* examined at The New York

Botanical Garden from ten European mycologists, six were *H. coralloides* as here defined and four were *H. laciniatum*. The species concepts of *H. coralloides* and *H. laciniatum* as here employed are those of Banker.

Collected from August to October. Uncommon in Iowa. Widely reported from the United States. Many of these reports undoubtedly refer to *H. laciniatum*.

3. HERICIUM ERINACEUS (Fries) Pers. Myc. Eu. **2**: 153. 1825. (FIG. 13)

Hydnum Erinaceus Bull. ex Fries, Syst. Myc. 1: 407. 1821.
Medusina patula Chev. Gen. Env. Paris 1: 279. 1826.
Dryodon Erinaceus (Fries) Quél. in Cooke & Quél. Clav. Hymen. 198. 1878.
Manina cordiformis Scop. ex Banker, Mycologia **4**: 277. 1912.

Fructification a solid or porous, slightly flattened, massive body, laterally attached, bearing on the lower and outer portions long, downward-directed spines, soft fleshy, whitish, becoming warm-buff to various shades of brown in the herbarium; spines 1–4 cm. long, curved, terete, acute, more or less coalesced at the base, often merging to shorter, flexuous and sterile spines or hairs on the upper surface; hyphae 3–20 μ in diameter, with occasional clamp connections; gloeocystidia 5–9 μ in diameter, arising from the subhymenial region, conspicuous by the highly refractive content; basidia 25–40 $\times$ 5–7 μ, with 4 sterigmata; spores 5–6.5 $\times$ 4.5–5 μ, subspherical, smooth, hyaline, 1-guttulate.

The massive fructification with the long cylindrical spines clearly distinguishes this species. *Hydnum Caput-medusae* Bull. ex Fries is believed to represent a variation in which deformed spines are more apparent on the upper surface and the massive body shows some tendency of being made up of dividing and anastomosing processes.

Common in Iowa on living oak trees and dead wood of various frondose species from May to November. Widely reported from the United States.

DENTINUM S. F. Gray, Nat. Arr. Brit. Pl. 1: 650. 1821

Pileus with a central stipe, fleshy, white or pale; spores subspherical, white in mass. Growing on the ground.

DENTINUM REPANDUM (Fries) S. F. Gray, Nat. Arr. Brit. Pl. **1**: 650. 1821. (FIG. 9)

Hydnum repandum L. ex Fries, Syst. Myc. **1**: 400. 1821.

Tyrodon repandus (Fries) Karst. Rev. Myc. **3**: 19. 1881.

Sarcodon repandum (Fries) Quél. in Cooke & Quél. Clav. Hymen. 196. 1878.

Hydnum umbilicatum Peck, Bull. N. Y. State Mus. **10**: 953. 1902.

Hypothele repanda (Fries) Banker, Torreya **4**: 113. 1904.

Pileus up to 12 cm. in diameter, usually convex, sometimes depressed, irregular, uneven, fleshy, soft, cartridge buff to pinkish cinnamon when fresh, becoming warm buff to cinnamon-buff and wrinkled when dry, with slight odor and taste, gregarious; margin repand; stipe smooth, solid, 7 cm. or less in length, color and texture as in pileus; spines 1–6 mm. in length, 2–4 per mm., soft, fleshy, subulate to occasionally flattened, slightly decurrent as short warts; hyphae 3–12 μ in diameter, thin-walled, with scattered clamp connections; basidia 25–40 $\times$ 5–9 μ, clavate, with 4 sterigmata; spores 7–9 $\times$ 6.5–7.5 μ, obovate to subspherical, with a prominent apiculus, smooth, white in mass.

Hydnum repandum is recognized by the pallid, fleshy fructification which occurs strictly on the ground, and by the large obovate spores. It varies considerably in size, color and character of the spines.

Collected from August to October on the ground; common in Iowa. Its occurrence is widely reported throughout the United States.

CALODON Quél. in Cooke & Quél. Clav. Hymen. 196. 1878

With central stipe, fibrous, tough, dark colored; spores subspherical, echinulate to coarsely angular, subhyaline to brown. Growing on the ground.

KEY TO THE SPECIES OF CALODON

1. Pileus and teeth white to grayish brown; spores subhyaline. ..... 1. *C. amicum.*
1. Pileus and teeth darker; spores distinctly brown ..... (2)
   2. Pileus mostly convex or plane, not zoned, composed of two thick and very conspicuous layers, a spongy upper and a hard, compact lower layer; spores 5–6.5 μ in diameter ..... 5. *C. velutinum.*
   2. Pileus depressed in the center or infundibular, usually more or less zonate, duplex texture absent or less pronounced; spores 4.5–6 $\times$ 3.5–4.5 μ ..... (3)

3. Pileus distinctly zonate, occasionally roughened, without a reddish liquid. 2. *C. zonatum.*
3. Pileus weakly zoned; more roughened.....(4)
4. Surface finely pubescent; with a reddish liquid.....3. *C. ferrugineum.*
4. Surface glabrous, without a reddish liquid.....4. *C. scrobiculatum.*

1. CALODON AMICUM Quél. Assoc. Fr. Av. Sci. Compte Rendu **12**: 504. 1884. (FIG. 11)

Hydnum amicum Quél. Grevillea **8**: 115. 1880.

Hydnum vellereum Peck, Ann. Rep. N. Y. State Mus. **50**: 110. 1897.

Phellodon vellereus (Peck) Banker, Mem. Torrey Club **12**: 168. 1906.

Phellodon amicus (Quél.) Banker, Mycologia **5**: 62. 1913.

Pileus 12 cm. or less in diameter, single or confluent, irregular, channeled, usually depressed at the center, tomentose, subzonate, with a broad, whitish marginal zone and brownish toward the center, composed of a soft, felty upper layer and a tough, compact lower layer; odor when fresh very disagreeable; taste slight; stipe 1–4 cm. long, 0.5–2 cm. in diameter, uneven, varying greatly in size, composed of a soft, spongy surface layer and a hard, woody axial region, color of the older portions of the cap; spines 2–4 mm. in length, 4–6 per mm., crowded, subulate, terete, white when fresh, becoming light gray to grayish brown when dry; hyphae 3.5–6 μ in diameter, firm-walled, faintly colored under the microscope, without clamp connections, with slightly thicker walls in the compact regions; basidia 20–30 $\times$ 4–5 μ; spores 3.5–4.5 μ in diameter, spherical, coarsely echinulate, subhyaline or faintly colored.

Specimens of *Phellodon vellereus* determined by Banker and *P. amicus* and *Hydnum amicum* by Banker and Patouillard at The New York Botanical Garden are quite like our Iowa specimen. *Hydnum putidum* is similar if not identical.

Rare in Iowa. On the ground in October. Reported from Washington and from many states in the eastern United States.

2. CALODON ZONATUM (Fries) Quél. in Cooke & Quél. Clav. Hymen. 197. 1878. (FIG. 15)

Hydnum cyathiforme Bull. ex Fries, Syst. Myc. **1**: 405, in part. 1821.

Hydnum concrescens Pers. Myc. Eu. **2**: 164, in part. 1825.

Hydnum zonatum Batsch ex Fries, Epicr. 509. 1838.

Hydnellum zonatum (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. **5**: 27. 1879.

Phaeodon zonatus (Fries) Schröt. Krypt.-Fl. Schles. **3**[1]: 458. 1888.

Hydnellum concrescens (Pers.) Banker, Mem. Torrey Club **12**: 157. 1906.

Pileus 1–7.5 cm. in diameter, with a central stipe, gregarious, often confluent, depressed in the center, sometimes subinfundibular, distinctly zoned, with radiating ridges or occasionally rugged, pitted and less zoned, tough, fibrous, usually light colored at the margin to pecan brown or vandyke brown at the center; stipe short, 0.5–2 cm. in length, 0.5–2 cm. in width above, wider and with a spongy, bulbous base below, color similar to the pileus; teeth slender, terete, 1–3 mm. or less in length, 5–6 per mm., vandyke brown; hyphae 2.5–6.5 μ in diameter, colored under the microscope, without clamp connections; basidia 20–35 $\times$ 5–6 μ; spores 4.5–6 $\times$ 3.5–4.5 μ, subspherical, coarsely tuberculate, russet brown in mass.

Calodon zonatum seems to be closely allied to *C. ferrugineum* and *C. scrobiculatum*. Banker (1913) regards the latter two names as synonyms. Since these are maintained as distinct by more recent writers, as Rea (1921), Bourdot and Galzin (1927) and other European workers, it seems best to keep them separate for the present.

Fairly common in Iowa; on the ground from August to November. Widely reported from the United States.

3. CALODON FERRUGINEUM (Fries) Quél. in Cooke & Quél. Clav. Hymen. 196. 1878.

Hydnum ferrugineum Fries, Syst. Myc. **1**: 403, *non* Persoon. 1821.

Hydnellum ferrugineum (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. **5**: 27. 1879.

Phaerodon ferrugineus Schröt. Krypt.-Fl. Schles. **3**[1]: 459. 1888.

Hydnellum sanguinarium Banker, Mem. Torrey Club **12**: 152. 1906.

Generally distinguished from *C. zonatum* by the thicker and less zonate pileus, the whitish margin, the mass of tubercles in the middle and the reddish liquid. The two forms are similar and differ little in the microscopic characters. The flesh pink

or onion-skin pink margin of *C. zonatum* described by Coker suggests the pale margin of *C. ferrugineum*. After years of study and observation of fleshy hydnums, Banker apparently came to regard the red juice as having little weight in the separation of species.

Collected on rich humus in woods. September. Uncommon in Iowa. Widely reported from the eastern United States.

4. CALODON SCROBICULATUM (Fries) Quél. in Cooke & Quél. Clav. Hymen. 197. 1878.

Hydnum cyathiforme Bull. ex Fries, Syst. Myc. **1**: 405, in part. 1821.

Hydnum scrobiculatum Fries, Epicr. 509. 1838.

Hydnellum scrobiculatum (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. **5**: 27. 1879.

Generally separated from *C. zonatum* by the more rigid, thicker, less zonate and rougher pileus. Bourdot and Galzin indicate that the fungus may be separated from *C. ferrugineum* by the smoother surface and the absence of a purplish liquid. Banker, however, regards *C. ferrugineum* and *C. scrobiculatum* as representing the same species. It seems equally plausible that *C. scrobiculatum* and *C. zonatum* represent one species. Many specimens under both names were studied at The New York Botanical Garden. They clearly merge and are practically indistinguishable. Careful study of living material under natural conditions and in culture if possible seems highly desirable in order to determine satisfactorily the validity of these similar species.

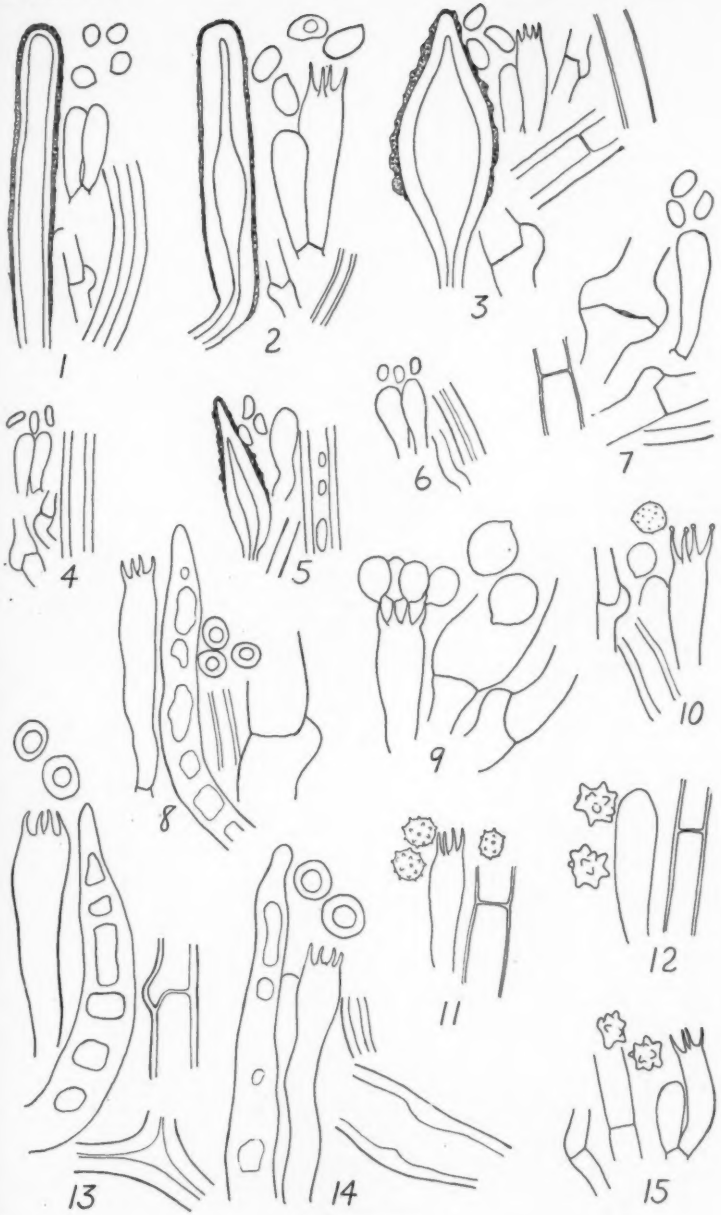
5. CALODON VELUTINUM (Fries) Quél. in Cooke & Quél. Clav. Hymen. 197. 1878. (FIG. 12)

Hydnum velutinum Fries, Syst. Myc. **1**: 404. 1821.

Hydnellum velutinum (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. **5**: 27. 1879.

Hydnum spongiosipes Peck, Ann. Rep. N. Y. State Mus. **50**: 111. 1897.

Pileus 2–12 cm. in diameter, with a central stipe, convex, plane or occasionally depressed at the center, not zoned, finely tomentose, filled with a colorless or reddish liquid, with a soft felty



HYDNACEAE

upper layer and a hard compact lower layer, uniformly cinnamon brown; stipe 1–6 cm. long, 0.8–1.5 cm. in diameter above, becoming wider toward the base, with a compact, hard central core surrounded by a soft felty layer, color of the pileus; spines 1–6 mm. in length, 2–4 per mm., subulate, terete, acute, color of the pileus; hyphae 3–6 μ in diameter, fairly thick-walled, without clamp connections, seldom over 4.5 μ in diameter in the spines, brownish in color; basidia 24–45 $\times$ 5–8 μ; spores 5–6.5 μ in diameter, spherical, coarsely tuberculate, brown.

Collected in August and September on the ground; uncommon in Iowa. Its occurrence is widely reported throughout the United States.

DEPARTMENT OF BOTANY,
STATE UNIVERSITY OF IOWA,
IOWA CITY, IOWA

EXPLANATION OF PLATE 33

All figures are drawn with camera lucida at a magnification of 1650 diameters, reduced to $\times$ 1000 in reproduction.

Hyphae, basidia and spores are shown in each figure. Cystidia are included also in figures 1, 2, 3 and 5, and gloeocystidia in 8, 13 and 14.

Fig. 1, *Steccherinum ochraceum;* 2, *S. setulosum;* 3, *S. septentrionale;* 4, *S. adustum;* 5, *S. rawakense;* 6, *S. pusillum;* 7, *S. pulcherrimum;* 8, *Hericium laciniatum;* 9, *Dentinum repandum;* 10, *Auriscalpium vulgare;* 11, *Calodon amicum;* 12, *C. velutinum;* 13, *Hericium Erinaceus;* 14, *H. coralloides;* 15, *Calodon zonatum.*

NEW AND RARE MYCETOZOA FROM LONG ISLAND

ROBERT HAGELSTEIN

(WITH PLATE 34)

In studying extensive collections of Mycetozoa made on Long Island in recent years, I have come across a form of *Comatricha* which I propose as a new species.

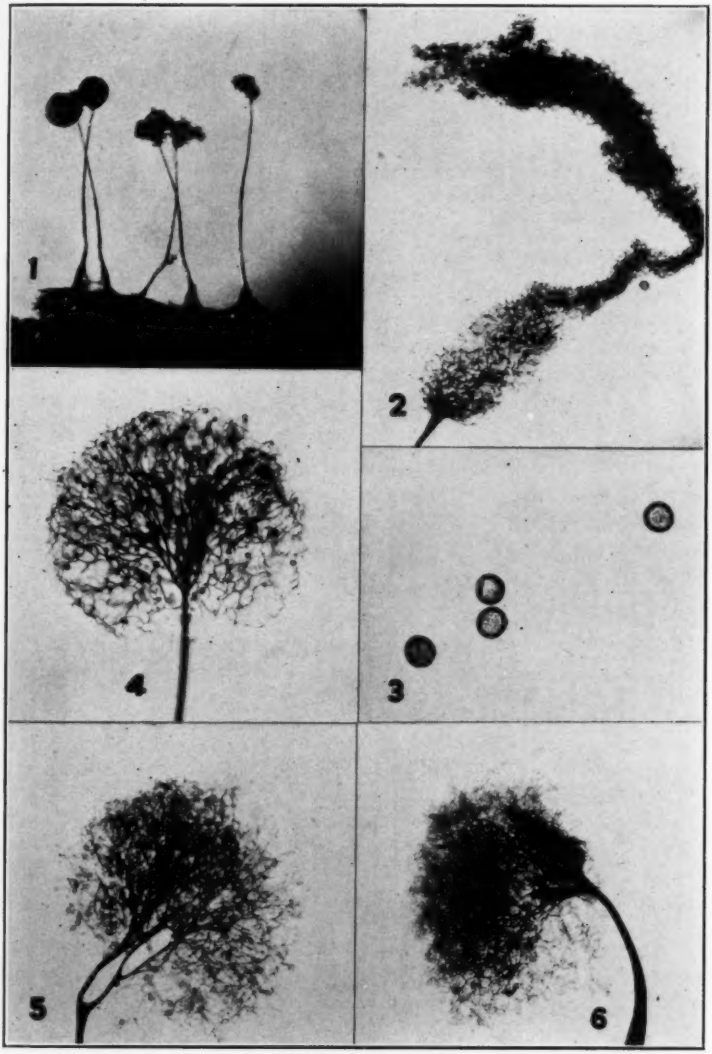
Comatricha extendens sp. nov.

Sporangiis gregariis, globosis, fuscis, stipitatis, ad 4 mm. altis; stipite subulato-gracile, atro, nitente; columella nulla; capillitio rete intricatum formante, elastice protrudente; sporis violaceo-brunneis, minutissime echinulatis, 8.5–10 μ diam.

Plasmodium? Total height 2 to 4 mm., unexpanded. Sporangia globose, stalked, gregarious, 0.4 to 0.65 mm. diam., dark purplish-brown; sporangial wall firm, finally evanescent. Stalk subulate, slender, black, shining, from 4 to 6 times the size of the sporangial body, rising from a distinct hypothallus; the stalk either expanded at the top or splitting into several parts, as many as eight, which form the primary branches of the capillitium. Columella lacking or obsolete. Capillitium a tangled mass of anastomosing purplish-brown threads springing directly from the stalk at the base of the sporangium; threads stouter at the base, becoming uniformly more slender, branched, somewhat looped or netted, but without a surface net; capillitium more or less elastic, ultimately expanding into a cylindrical plume 2 or 3 times the size of the sporangial body. Spores brownish-violet, minutely but distinctly spinulose, 8.5 to 10 μ diam.

Habitat. Indoors, on the lower side of flooring in close proximity to steam pipes; in a moist environment with temperature 90 to 100 degrees Fahr. Mitchell Field, Long Island, N. Y., March 1930.

The development is perfectly matured and uniform throughout, and does not appear to be abnormal. Except for the lacking columella, replaced by an extending capillitium attached directly to the top of the stalk, the form is like *Comatricha nigra* (Pers.)



COMATRICHA SP.

Schröt. The absent columella would indicate a genus separated from *Comatricha* as the columella is an important feature of the latter. I am convinced, however, that *C. extendens* has developed from *C. nigra* through *Comatricha elegans* (Racib.) List., and have specimens of *C. elegans* from Mitchell Field, where, instead of continuing into the sporangium as a columella, the stalk divides into two or more branches below the sporangial base, with the capillitium retaining its globose shape. These forms, in my opinion, are intermediate between *C. elegans* and *C. extendens*, but nearer *C. elegans*. From the same locality, a series of intermediate forms connect *C. elegans* with *C. nigra*.

The warm, moist environment of the habitat suggests that the species may be looked for in the tropics. Mitchell Field is an army aviation post, and in the past, large quantities of tropical woods have been brought there for various purposes.

It is noteworthy to record also the collection of *Comatricha lurida* List. which, so far as I know, has not been reported from North America. The species was found twice, on leaves, at the Albertson kettle hole, Long Island, in July 1927. The specimens have globose, light brown sporangia, on short slender stalks. The columella extends about half way into the sporangium, and divides at the top into the primary branches of the capillitium. While some of the threads are bent downwards, there are no attachments to the lower part of the columella, nor is there any firm, peridial base, with attached threads, as in *Comatricha rubens* List., a closely allied species. The spores are light violet-gray in color, irregularly globose, distinctly warted, and measure about 7 μ.

MINEOLA, NEW YORK

EXPLANATION OF PLATE 34

Fig. 1–3, *Comatricha extendens:* 1, group of sporangia, × 10; 2, top of stalk and capillitium, × 50; 3, spores, × 500; 4–5, *Comatricha elegans* (Racib.) List. from Mitchell Field; 4, capillitium, × 100; 5, intermediate form approaching *Comatricha extendens*, capillitium, × 100; 6, *Comatricha lurida* List. from Long Island, capillitium, × 100.

A NEW SAPROPHYTIC SPECIES OF LAGENIDIUM, WITH NOTES ON OTHER FORMS

JOHN N. COUCH

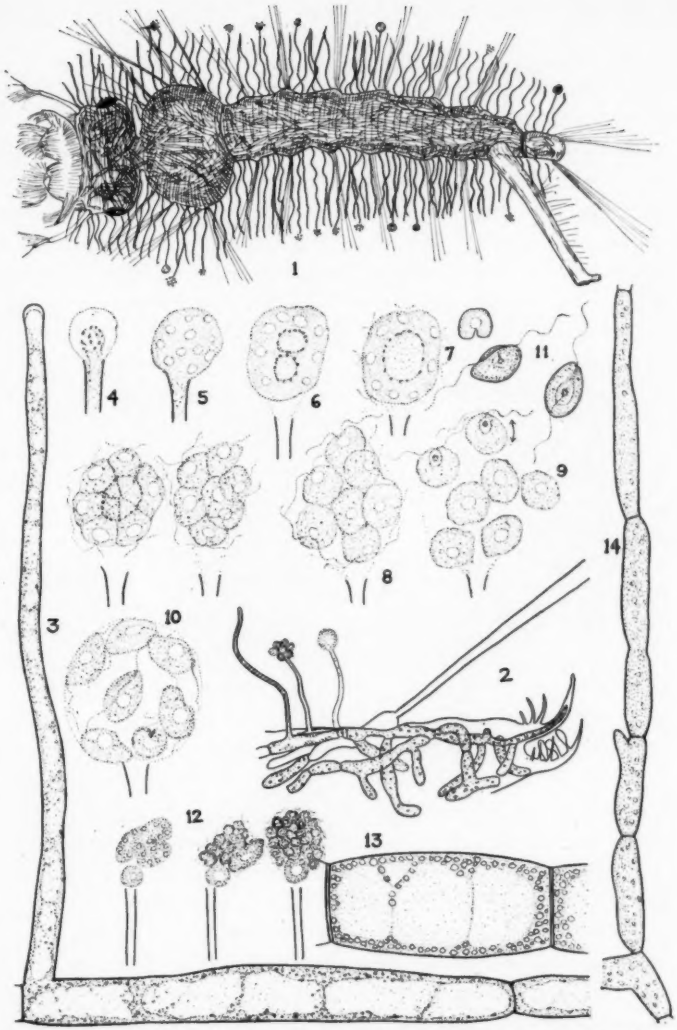
(WITH 40 TEXT FIGURES)

Up to the present time so far as known no species of the Ancylistales has been grown in pure culture either on solid or in liquid media. The new species described below, while a facultative parasite on mosquito larvae, *Daphne* and copepods, can be cultured on a variety of nutrient agars. It is also noteworthy because it probably represents a connecting link between these two lower orders of non-filamentous fungi and the filamentous Phycomycetes.

To date about fifteen species of *Lagenidium* have been described, only three of which have been reported in the United States and these from the northeastern states. In the present paper, in addition to the new species, four species known heretofore only from Europe are reported in America, as well as another form, the specific identity of which is doubtful.

Lagenidium giganteum sp. nov. (FIGS. 1–19)

The main hyphae are segmented, being constricted or not constricted at the septum, the segments sometimes separating from each other; branched. When growing on a copepod, *Daphne*, or mosquito larva, the large segmented hyphae are within the host, but numerous delicate hyphae extend from the host for a distance of one or two millimeters to form a fringe which has much the appearance of a delicate species of *Aphanomyces*. Hyphae 6–40 μ thick, the segments 50–300 μ long. The hyphal walls contain cellulose giving a purplish reaction with chlor-iodide of zinc. The protoplasm has the pale whitish gleam as in the Ancylistales. Any segment may become a sporangium. The sporangium empties its content in an undifferentiated, naked mass (or sometimes several masses) through a tube, the dimensions of which are 6–10 $\times$ 50–300 μ. This mass becomes differentiated into a variable



FIGS. 1–14. *Lagenidium giganteum:* 1, mosquito larva parasitized by *Lagenidium* (note large, internal, segmented hyphae and delicate external threads most of which are long emergence tubes for sporangia), × about 14; 2, post-abdominal region of *Daphne* showing fungus within, × about 135; 3, sporangium with emergence tube, × 625; 4–10, discharge of sporangial contents and formation of spores, × about 570; 11, spores in motile state, × about 570; 12, sporangium from which contents were discharged in three separate lumps, × about 230; 13, hyphal segment with large vacuoles, × about 625; 14, sketch of part of a hypha, × 325.

number of laterally biciliate zoöspores. Zoöspores 8–9 × 9–10 μ, their movement as in *Achlya* but rather sluggish. Monoplanetic. Sexual reproduction not observed.

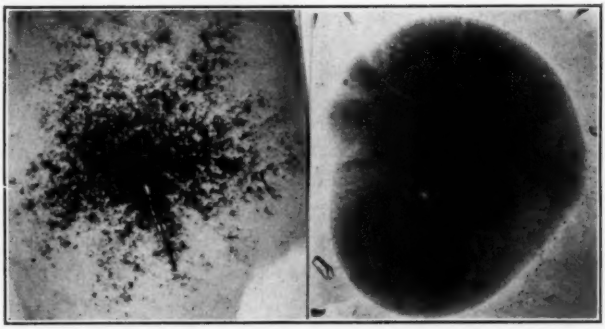
Weakly parasitic on mosquito larvae, copepods and *Daphne*. Also culturable as a saprophyte. This fungus first appeared on *Daphne* and copepods in material collected from the lake at Mountain Lake, Va., during the summer of 1933. Its remarkable characters were noted at the time and some drawings and notes were made. A few weeks later the same fungus was again found on mosquito larvae at Chapel Hill, N. C., and this time was isolated in pure culture. It appears to be more closely related to *Lagenidium* than to any other known fungus, and due to its unusually large size I am describing it as *L. giganteum*.

The fungus was isolated in pure culture by picking up with a platinum loop a drop of water containing some of the discharged zoöspores and smearing this drop over a corn meal agar plate. After the spores had germinated, several of the germlings were cut out under the binocular microscope and planted separately on fresh, corn meal agar plates.

Growth proved to be exceedingly slow and so attempts were made to find a better culture medium. On corn meal agar the culture attained a diameter of 0.7 cm. in 5 days, the threads being rather slender and considerably coiled. On corn meal agar slightly alkaline, about pH 7.5, the growth was about 1 cm. in diameter after 5 days, the threads being rather thick. No growth was obtained when the alkalinity was increased beyond pH 7.5 nor when the culture medium was acidulated with phosphoric acid. In 1 per cent peptone growth very poor; in 1 per cent beef extract (Difco) growth excellent, about 2 mm. diameter after 3 days, hyphae of uniform diameter, not constricted at septa. In equal parts of 1 per cent peptone, 1 per cent maltose, 1 per cent meat extract, growth fair, about 1.5 mm. diameter after 3 days, hyphae with numerous swellings. On maltose peptone agar (2 per cent agar, 0.3 per cent maltose, 0.1 per cent meat peptone) the threads were straight, the hyphae being constricted at the segments. On corn meal agar the hyphae were coiled but not constricted at the segments.

Soon after the fungus was isolated in pure culture on maltose

peptone agar a sector of growth appeared that was easily recognizable with the unaided eye as being distinct. The new type of growth was composed of threads 8–20 μ thick, mostly 12 μ thick. The threads branched irregularly, contained few septations, and formed an open type of growth. On the same medium the old type of growth was composed of compactly arranged, much septate, rarely branched, straight threads. The two types of growth have been cultured separately for several months, both retaining their individual growth characters as shown in the photographs (FIG. 15, 16).



FIGS. 15 AND 16. *L. giganteum*. Two strains growing under the same conditions on maltose-peptone agar, original strain on right, mutant on left, × about 2½.

Each segment may form a sporangium. A long emergence tube grows out from each segment, the contents of which, however, show no differentiation into spore origins. The wall at the tip of the emergence tube thickens and softens, finally giving way to the pressure within. First there emerges a globose mass of hyaline material and the granular protoplasm flows out into this. Apparently the very delicate vesicle which later can be seen to surround the developing spores is derived from this substance. This mass of protoplasm is globose or more usually subglobose and contains numerous, small, contractile vacuoles. At this stage the protoplasm may show slight twitching movements. Suddenly one or two vacuoles appear in the center of the mass. These grow rapidly in size, fusing, if more than one is present, to

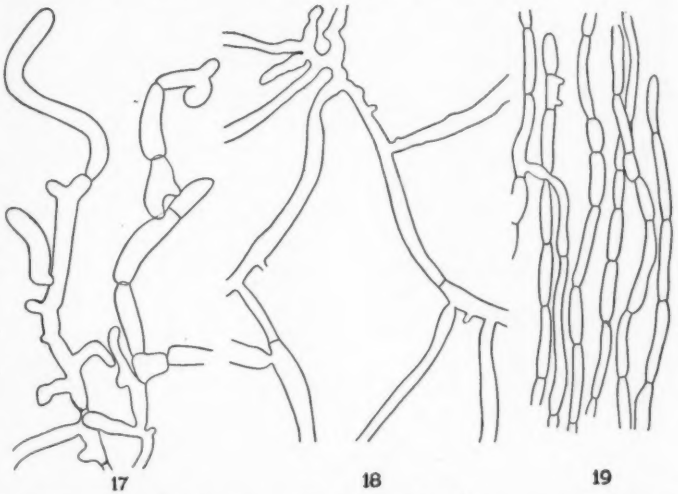
form the conspicuous central vacuole. This central vacuole is lined with small but conspicuous granules. The spore origins now become evident, each with a small distinct vacuole just opposite which two cilia stubs appear in the cytoplasmic membrane. Simultaneously cleavage furrows appear, extending outwards from the large central vacuole. The whole mass at this stage rocks and jerks feebly as a result of the motion of the young cilia. Suddenly the central vacuole disappears, the spore mass shrinking, and the spores sticking together motionless for several minutes. The spores then begin rounding off, separate from each other, and about 3–5 minutes after the clumping stage, begin rocking and jerking again. Within 8 to 10 minutes more the spores change from irregular rounded masses to the typical "kidney-bean" shaped spores. A few seconds after the spores have reached maturity the delicate vesicle is broken and the spores swim away. In most cases I have been unable to see a vesicle.

Frequently the contents of the sporangium may emerge and form not one spherical mass of protoplasm but several masses, in which event the development of the zoöspores will be progressive, the mass of protoplasm which emerged first forming its spores first, etc.

No detailed studies on the development of the sporangia of *Lagenidium* have yet been published but from my observations on *L. Oedogonii* it appears that development in that species is similar to that described here. The noteworthy feature is the large central vacuole in the discharged mass of protoplasm which seems to play the same rôle here as in the sporangium of *Saprolegnia*. In *Pythium*, however, there is no large vacuole in the discharged mass of protoplasm.

Lagenidium giganteum occupies a unique position in the Ancylistales. All the other species so far described are obligate parasites, so far as known, and have a rudimentary mycelium. The present species would seem to be intermediate between the filamentous Phycomycetes and the non-filamentous forms. It may be cultured on a variety of media, on some of which an extensive mycelium is developed. In spite of the wide discrepancies between this species and the other members of the genus *Lagenidium* it would seem best to place this species in *Lagenidium*, at least until the sexual stages can be found.

A few experiments have been carried out to see if this fungus would kill healthy mosquito larvae. Two large, healthy larvae were placed in Petri dish containing spring water, and *Lagenidium* in spore-forming state was added Dec. 13, 10:30 A.M. On Dec. 14 one of the larvae was seen eating the fungus. On Dec. 16 both larvae were alive. On Dec. 17, 2 P.M., one larva was quiet

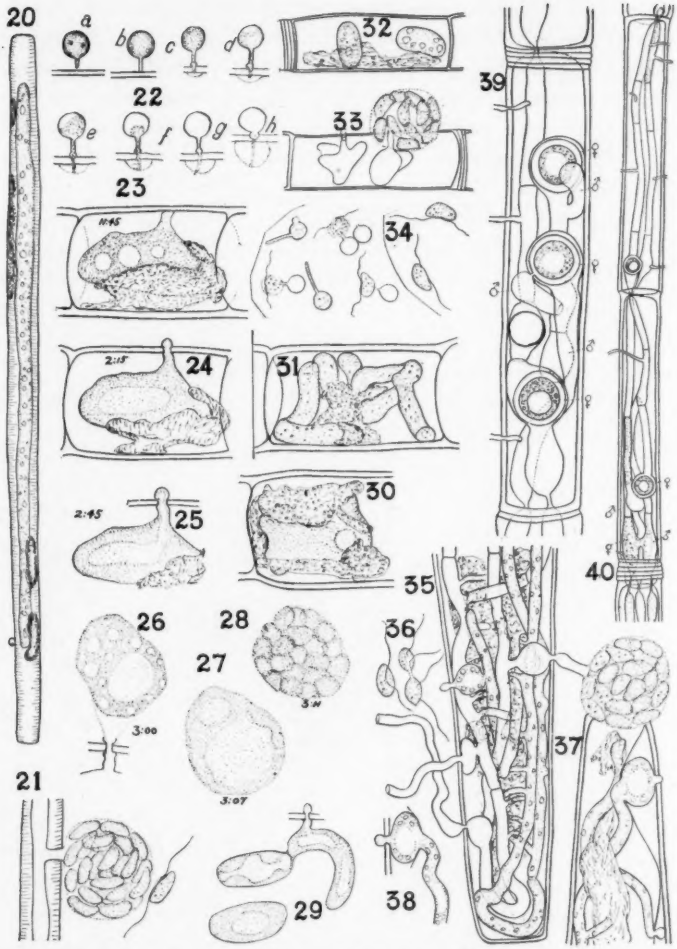


FIGS. 17–19. *L. giganteum*. 17, sketch of hyphae on corn meal agar; one of earliest cultures, × 140; 18, sketch of threads from mutant, strain from fig. 15 (note open irregular growth, few septations and thicker hyphae); on maltose-peptone agar, × 110; 19, sketch of hyphae from original strain (note compact, regular growth and abundant regular septations); on maltose-peptone agar, × 110.

(almost dead), infected in region of eyes, other infected near tuft of hairs on head. Essentially same results obtained in another experiment in which 7 larvae were used. Whether the fungus killed the larvae or whether the larvae become weakened and were then parasitized, I cannot yet say.

LAGENIDIUM CLOSTERII de Wildeman. (FIGS. 35–38)

Thallus composed of long, narrow, straight or much twisted, branched threads. Threads sometimes of more or less uniform diameter throughout (about 1.8–2.8 μ thick), sometimes swollen



FIGS. 20 AND 21. *Lagenidium brachystomum* Scherffel. 20, elongated, worm-like, non-septate thallus in cell of *Synedra* sp., drawn 11:30 A.M.; 21, spore formation, 6 P.M., in same thallus, spores surrounded by bladder; on right, a biciliate zoöspore, both figures × about 400.

FIGS. 22–31. *Lagenidium Oedogonii* Scherffel. 22 a–g, stages in the germination and penetration of a zoöspore, the process occupying about an hour and a half, slightly diagrammatic (note appressorium and callus on inside of host wall); h, a spore, with appressorium, whose germ tube has

and of quite irregular diameter. Hyphal contents of characteristic appearance with numerous, large, shiny, irregular bodies, and with pale cytoplasm. Thallus when mature breaking up into units, each of which may form a sporangium. From the sporangium arises a short branch which enlarges at the end to form a spherical body. From this body there arises a fine thread which penetrates through the wall of the *Closterium* and may grow out for some distance (20–30 μ) away from the host. The sporangium discharges its contents into a vesicle in which zoöspores are differentiated. Zoöspores biciliate, about 3.8 × 5.6–6.3 μ in the motile state, shaped as in *Pythium*, with several shiny globules. The spores swim with a peculiar, hopping motion, sometimes however they swim in a smooth, regular, spiral path, as is characteristic of the zoöspores of *Achlya*, *Pythium*, etc. Sexual reproduction not observed.

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successfully penetrated host wall (note large callus), × 675; 23–28, stages in late development of sporangium: 23, note granular cytoplasm, vacuoles and hyaline, emergence tube flattened out against host wall; 24, large, central vacuole and tube penetrated through host wall; 25, note central vacuole extending out into tube; 26, content of sporangium discharged (note large vacuole and numerous smaller ones); 27 and 28, stages in spore formation; in fig. 28 there are very faint signs of a bladder; about seven minutes after spores became distinct, bladder broke and spores dispersed; 29, above, a sporangium in which spore origins (?) have appeared and below five minutes later the origins (?) have disappeared, leaving large central vacuole with a smooth outline; 30 and 31, sporangia both of which emptied a few hours after sketches were made; figs. 23–31 × about 480.

FIGS. 32–34. *Lagenidium* sp.: 32, two young sporangia in *Oedogonium* cell; 33, two sporangia, one of which has just discharged its content which has now become organized into spores (note bladder); 34, two spores on right in motile condition just after bladder broke; on left a group of spores kept under observation for about three hours, some spores germinating by delicate tube, others emerging from cysts (note delicate bladder around spores which have just emerged from cysts); one spore is shown which had begun to germinate but which reversed its action and is emerging from cyst, all × about 625.

FIGS. 35–38. *Lagenidium Closterii* de Wildeman: 35, part of a *Closterium* cell nearly filled with parasites (note striking resemblance between young threads and *Pythium*); on right discharged content of sporangium is being differentiated into spores within a bladder; 36, three spores, two of which are joined by a narrow band of cytoplasm; 37 and 38, formation of exit tube of sporangium, all × about 560.

FIGS. 39 AND 40. *Lagenidium Marchalianum* de Wildeman: 39, several oögonia and antheridia within *Oedogonium*, × about 625; 40, long threads of parasite, young and empty sporangia, and oögonia and antheridia and one parthenogenetic egg, × about 335.

Collected only once (No. 6, April 23, 1933) in roadside ditch near Wilmington, N. C., on *Closterium* sp. Cell membrane containing cellulose and giving a lavender color with chlor-zinc-iodide. Found once in Bohemia by Karel Cejp (Bull. International de l'Académie des Sciences de Bohême *pl. 1 & 2.* 1933).

The elaborately developed mycelium of this parasite in the early stages before it breaks up into sporangia, may easily be confused with that of certain species of *Pythium* parasitic on algae. The large shiny granules and the clear cytoplasm easily distinguish the thallus from that of *Pythium.*

LAGENIDIUM MARCHALIANUM de Wildeman. (FIGS. 39, 40)

Thallus composed of elongated, segmented, sometimes branched threads which may extend throughout the length of several cells of *Oedogonium* sp. Threads 2.2–6.7 μ thick, slightly or not at all constricted at the segments, or sometimes swollen at the segments, more or less straight, but often irregular, sometimes confined to one cell; threads narrowly constricted when passing through the cross walls of the host, about 1 μ thick. Segments 30–60 μ long. Each segment may become a sporangium, giving rise to a very delicate (1.5–2 μ thick), short exit tube which pierces the host wall, usually extending out only 4–5 μ beyond the host wall. Spore discharge not observed. Oögonia and antheridia abundant. Oögonia usually intercalary, rarely terminal, formed by the enlargement of one of the segments, up to 20 μ thick; eggs 8–14 μ, with very thick, smooth walls; antheridia may arise by the enlargement of the cell adjacent to the oögonium, this cell being directly transformed into an antheridium or the cell adjacent to the oögonium may branch to form the antheridium, or the antheridium may arise from the cell of another thread. Only one antheridium to an oögonium and sometimes the egg may be formed parthenogenetically. Antheridium emptying its entire contents through a small tube into the developing egg.

Collected only once on a large species of *Oedogonium*, July 1933, in the lake at Mountain Lake, Virginia. This species shows a close relationship to *L. Rabenhorstii* in the appearance of the mycelium, sporangia, oögonia and antheridia. However, that species, with one exception (Graff, Mycologia **20**: 169, 1928, who

reports it on *Oedogonium plusiosporum* Wittr.), has been reported only on filamentous forms of the Conjugales, the mycelium is limited to one cell, and the eggs are from 15–20 μ thick, considerably larger than in the present species.

Of the several species described on *Oedogonium*, *L. Oedogonii* Scherffel, *L. Zopfii* de Wildeman, *L. syncytiorum* Klebahn, *L. Marchalianum* de Wildeman, it seems, from a comparison with the original descriptions and figures, that the present species is closest to *L. Marchalianum*. That species has a mycelium the threads of which are about 4–5 μ thick and composed of more or less cylindrical segments. The threads are branched and extend through as many as six or seven host cells. Sexual reproduction not observed. The present species differs from *L. Zopfii* in having a smooth egg membrane while in *L. Zopfii* the membrane is minutely rough. It may be that these three species are the same but the evidence at hand at present does not justify such a conclusion.

LAGENIDIUM BRACHYSTOMUM Scherffel. FIGS. 20, 21)

Thallus composed of a long, unbranched, non-septate, delicately walled tube, 4–7.5 $\times$ 150–250 μ, with whitish gleaming protoplasm and shiny fat (?) globules. The entire tube becomes one sporangium which empties its contents through a short neck. Spores formed within a vesicle, laterally biciliate, 4 $\times$ 6 μ when swimming. Sexual reproduction not observed.

Collected only once in *Synedra* sp., Feb. 18, 1932, Chapel Hill, N. C.

Of the three species of *Lagenidium* (*L. Cyclotellae* Scherffel, *L. enecans* Zopf, and *L. brachystomum* Scherffel) which have been described on diatoms the present species seems to agree best with *L. brachystomum* in having a simple, unbranched, non-septate thallus, and a short emergence papilla.

LAGENIDIUM sp.? (FIGS. 32–34)

In the same material in which the *Lagenidium brachystomum* appeared there was a very small parasite on *Oedogonium* which at the time I took to be the same as *L. brachystomum* on *Synedra* sp. Without infection experiments, however, the identity cannot be

determined with certainty. Thallus ovoid or somewhat irregular, with the whitish protoplasm and glistening fat bodies characteristic of the genus, 7–10 × 15–20 μ, non-septate. Spores surrounded by a vesicle the presence of which, though faint, could be attested by the fact that a spore came to rest on the vesicle before it broke. Spores laterally biciliate, about 4 × 7.5 μ in the motile condition, about 5 μ thick when encysted. Spores diplanetic, coming to rest after swimming for a short while, encysting and then emerging from cysts 1 to 3 hours later, to swim again in the laterally biciliate condition. Even the single spores which emerged from the cysts appeared to be surrounded by a delicate vesicle. In a few cases encysted spores which had begun to germinate emerged from the cysts leaving both germ tube and cyst empty. Sexual reproduction not seen.

Collected only once, February 18, 1932, Chapel Hill, N. C.

LAGENIDIUM OEDOGONII Scherffel. (FIGS. 22–31)

Sporangia within the cells of *Oedogonium*, usually one sporangium to a cell, rarely two. Irregularly ovoid, sometimes lobed, very rarely filamentous, 20–25 × 35–52 μ, the filamentous forms up to several hundred microns long and composed of a coiled thread. Protoplasm with a pale whitish gleam and with conspicuous shiny globules. Sporangium when just mature with numerous, small vacuoles; spore origins delimited within sporangium but quickly disappear, leaving a large, central vacuole. Exit tube short, extending through the host wall, and only a few microns beyond. Contents of sporangium emerging to form an irregularly globoid mass surrounded by a very indistinct vesicle; with a large central vacuole and numerous smaller ones. Spores laterally biciliate, pointed at the anterior and rounded at the posterior end. Spores swimming smoothly as in *Achlya*, rotating on their long axis and at the same time moving in a spiral path. Encysted spores about 6.6 μ thick.

This species, first described by Scherffel in Hungary, has been collected by me near St. Louis, Mo., on *Oedogonium*. The material from Missouri agrees in most details with Scherffel's except that the spores in his species were not surrounded by a bladder; also he found sexual reproduction, whereas I found none. Scherf-

fel describes the spores as being sometimes diplanetic. In such cases the spores are formed within the sporangium, emerging individually to collect in an irregular, spherical cluster at the tip of the emergence tube. Here they encyst, remain for some time, and later emerge from the cysts to swim in the laterally biciliate condition. Such spores are thus diplanetic as in *Achlya*. This species is very distinct from other species of *Lagenidium*, being characterized by the irregularly ovoid, non-segmented thallus.

SUMMARY

A new species of *Lagenidium* is described as *L. giganteum*. It is a facultative parasite on mosquito larvae, *Daphne*, and copepods. The development of the sporangia is described. Sexual reproduction was not observed. It is also saprophytic, being culturable on a variety of nutrient agars. Soon after the fungus was isolated on agar a mutation occurred. The mutant can be recognized by the more irregular growth, thicker hyphae, and fewer septations. Both the mutant and the original strain have been kept in culture for over a year. This fungus probably represents a connecting link between the non-filamentous Phycomycetes and the filamentous forms. *Lagenidium Closterii*, *L. Marchalianum*, *L. Oedogonii* and *L. brachystomum* are described from America for the first time. A doubtful species of *Lagenidium* also is described.

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LITERATURE

Atkinson, G. F. Some fungus parasites of algae. Bot. Gaz. **48**: 321–338, *fig. 1–8*. 1909.

Fitzpatrick, H. M. The lower fungi. 1930.

Minden, N. von. Kryptogamenflora der Mark Brandenburg **5**: 433–440. 1911.

Scherffel, A. Endophytische Phycomyceten-Parasiten der Bacillariaceen und einige neue Monadinen. Archiv für Protistenkunde **52**: 1–141. *5 pl.* 1925.

Wildeman, E. de. Notes Mycologiques. Ann. Soc. Belge Micr. **21**: 5–31. *Pl. 1, 2*. 1897.

A COMPARATIVE STUDY OF CERTAIN SPECIES OF MARASMIUS AND COLLYBIA IN CULTURE [1]

JEAN D. ARNOLD

(WITH PLATES 35 AND 36)

INTRODUCTION

During recent years considerable attention has been devoted to the subject of sexuality in the Hymenomycetes, with the result that conditions prevailing in many species have been determined. In the Agaricaceae the genus *Coprinus* has received the most study, but species of *Hypholoma*, *Panaeolus*, *Pholiota* and other genera have also been investigated.

It is well known to students of the Agaricaceae that some of the small members of the genus *Collybia* resemble the genus *Marasmius* very closely, not only in size and general appearance, but also in the ability of the dried pileus to revive when moistened. Since little attention has been given to species of *Marasmius* or the marasmioid species of *Collybia* with regard to their development and sexual behavior, several species of *Marasmius* and *Collybia* were used in the present study. The following species received the most attention in this work: *Marasmius elongatipes* Peck, *Collybia tuberosa* Fries, *C. cirrata* Fries and the form hitherto recognized as *C. cirrata* Fries var. *Cookei* Bres. (**1**).

HISTORICAL

As early as 1860 there is a record of simple experiments dealing with *Collybia tuberosa*. In "The Gardeners' Chronicle and Agricultural Gazette" for that year there is a mycological note written by Berkeley (**3**, p. 456). *Collybia tuberosa* is referred to there as *Agaricus tuberosus*, and Berkeley points out that the tubers them-

[1] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan. March 23, 1934. Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 545.

selves were once considered a distinct genus, and were known as *Acrospermum cornutum*. The last paragraph of the article reads as follows: "It may not be uninteresting to some of our readers to state that many of these tuber-like bodies will produce their true fruit if placed under proper conditions of air, light, heat, and moisture. We have ourselves shown this to be the case in one instance many years since, and Mr. Currey, who bids fair to follow in the steps of the Messrs. Tulasne in the study of Mycology, has been successful in several more or less important cases."

However, little interest was shown in the study of this group of fungi until 1918, when Mlle. Bensaude's thesis (**2**) was published in Paris. This outstanding piece of work on the life cycle and sexuality of the basidiomycetes aroused widespread interest. The history of subsequent investigations dealing with this general topic has been reviewed so thoroughly elsewhere (**9**) (**14**), that mention will be made here of only those studies relating to the species dealt with in this investigation.

Most of the information on hand at present concerning the species here involved is due to the investigations of Kniep (**12**) (**13**), who reported heterothallism in *Collybia cirrata* and *C. tuberosa* and the occurrence of haploid fruit bodies in the latter species.

Kniep (**14**, p. 413) also studied *C. cirrata* in more detail and found as the result of mating monosporous mycelia obtained from basidiospores from diploid fruit bodies, that this species was tetrapolar with regard to the segregation of its sexual factors. He also found that monosporous mycelia originating from basidiospores from two separate fruit bodies both collected from the same wooded area, were completely interfertile when paired in all possible combinations. Monosporous mycelia from the spores of each pileus, when paired with other monosporous mycelia of the same pileus, gave a clear case of tetrapolarity.

TECHNIQUE AND MEDIA USED

The original cultures obtained in the study of these species were all procured from fresh material, either from spores or from tissue from the pileus or sclerotia when these were present, using the customary precautions against contamination. When mono-

sporous cultures were made, Kauffman's method (11) for the isolation of single spores by spraying on agar plates was used, with the following modification: Each time a plate was sprayed, half the suspension in the capsule was poured out and the volume made up again using sterile distilled water. This process was repeated several times, thereby making a series of dilutions among which a plate was obtained in which the spores were sufficiently scattered so that single spores could be isolated with little difficulty.

For cutting out the spores, a nichrome wire inoculating needle, flattened and pointed so that it resembled a minute scalpel, proved very serviceable. As each spore was cut out it was transferred to a sterile agar plate. After about two weeks' growth, the cultures were examined for clamp connections. If none appeared, transfers were made to malt agar slants. All cultures were stored on shelves in diffuse light at a temperature of about 20° C.

At first Kauffman's nutrient agar as given by Lohman (16) was used in the spore germination tests. Although it was found to be a satisfactory medium, it was later given up in favor of plain malt extract agar, which gave excellent development and was more easily prepared. The following proportions were used in the preparation of the malt agar:

Malt extract	25 grams
Agar	20 grams
Distilled water to make	1 liter

This was sterilized in an autoclave for 25 minutes at 12 pounds pressure. If the malt extract is completely dissolved in the water before being placed in the autoclave, and if a fairly good grade of agar is used, filtering is not necessary for ordinary purposes. Other media used were: Kotila's agar (15), Poole's medium for developing sporophores in *Collybia dryophila* (20), Etter's medium (8) and certain modifications of the last two. The following combinations were found to be the most useful for inducing fruiting in the species of *Marasmius:*

Medium 5.

Powdered oak wood	40 grams
Malt extract	10 grams
Peptone	0.1 gram
Water	120 cc.

Medium 10.

Corn meal	50 grams
Agar	15 grams
Prune extract	1 liter
(75 g. prunes in 1 l. water)	

The media used for the growth and fruiting of the *Collybia* species required different constituents. On Kauffman's and Kotilla's agars their growth was slow and scanty. However, since *Collybia tuberosa* and *C. cirrata* both grow upon decaying fungous material, it seemed logical to incorporate such material into the media used for their culture. In the vicinity of Ann Arbor, Michigan, *C. cirrata* grows in great abundance upon shrivelled remains of *Boletus luteus*. Accordingly, large numbers of these polypores were gathered in the autumn and dried. When needed, the dried *Boletus* caps were cooked in a little water and used as a substratum. This was placed in glass capsules 10 cm. in diameter and 7 cm. deep, and sterilized in an autoclave for 20 minutes at 15 pounds pressure. All three species of *Collybia* fruited well on this medium, producing fruit bodies (PLATE 36, FIGS. 8, 9). A decoction was also made by gently boiling for about an hour some of the dried caps in a quantity of water just sufficient to float them. To each liter of the filtrate 15 grams of agar were added. The mycelia of the three species of *Collybia* grew moderately well on this medium (PLATE 36, FIG. 1). A modification of Kauffman's agar was also used. This included all the nutrient materials as given in Kauffman's formula (**11**), but *Boletus* extract was used instead of water. The fungi grew fairly readily upon this, but fruiting was reduced (PLATE 36, FIG. 2 AND 3). This medium was used in small Erlenmeyer flasks, and the cultures were grown both in the dark and in diffuse light. Various media using prunes or prune extract were tried, but none of the three species of *Collybia* would grow at all on any medium containing prune. Similar results were experienced by Miss Mounce (**17**) with *Fomes pinicola*. The medium which was found to be most satisfactory was discovered in an attempt to duplicate as nearly as possible, under aseptic conditions, the natural habitat of the fungi. This was called medium 19 and was prepared as follows:

Ordinary greenhouse soil was placed in the bottom of glass capsules to a depth of 1.5–2 cm. and saturated with water. A dried pileus of *Boletus luteus* (or several, if small) was then laid on top of the soil, and the glass cover placed on the capsule. No cotton was used with the glass cover, as it was found that the fungi thrive better with freer access of air. The capsules and contents were then sterilized in the autoclave at 15 pounds pressure for 30 minutes, and when cool were inoculated. All three species of *Collybia* grew well on this medium and produced fruit bodies generally a little larger than those found in nature. This medium was also used in flasks with fair success (PLATE 36, FIG. 7). It was found that when the soil was sterilized first in a dry oven at a temperature of 140–150° C., and the *Boletus* and water added later and subsequently sterilized in the autoclave as above mentioned, no growth of the fungi occurred.

RESULTS

GERMINATION OF BASIDIOSPORES. Good spore germination was obtained on Kauffman's nutrient agar and on plain malt extract agar in petri dishes at room temperature (approximately 20–21° C.) in the following species: *Marasmius alliatus* (Schaeff.) Schröt., *M. capillaris* Morg., *M. elongatipes* Peck., *M. epiphyllus* Fries, *Collybia cirrata* Fries, *C. cirrata* Fries var. *Cookei* Bres. and *C. tuberosa* Fries. *Marasmius elongatipes* and the three species of *Collybia* fruited more readily than the other species, with the result that these species were studied in greater detail.

HAPLOID AND DIPLOID MYCELIA; OIDIA. In *Marasmius elongatipes* it was somewhat difficult to recognize any macroscopic difference between haploid and diploid mycelia. As a rule, the haploid mycelia were flatter, lying closer to the agar than the diploid mycelia, which were more fluffy. These differences, however, are only relative. There was a decided difference in the rate of growth, the diploid mycelium sometimes growing twice as fast as the haploid under identical external conditions. Different strains of the same species may show pronounced variation. Some of the haploid mycelia of *M. elongatipes* are brown and felty in appearance, and other haploid mycelia in the same species are white and fluffy. The diploid mycelia also may be either

pure white or brown. This will be discussed in greater detail later. No oidia have been observed on either the diploid or the haploid mycelia of *M. elongatipes.*

In each of the three species of *Collybia* studied it is easy to distinguish between haploid and diploid mycelia. All the mycelia are white or slightly creamy in color, but the haploids generally have a more opaque and powdery appearance than the diploids. Sometimes the haploid mycelium lies close to the agar, but at other times it is deep and fluffy, and always very powdery. The diploid mycelium differs in presenting radiating strands of a rather coarse nature, which are very conspicuous. The difference between a typical haploid and a typical diploid mycelium in this group is well shown by plate 36, figure 10. Clamp connections on all the diploid mycelia of these *Collybia* species are large and easily seen. Here also, there is a noticeable difference in the rate of growth of haploid and diploid mycelia. In the culture shown on plate 36, figure 10, the large haploid mat (three weeks old) had a radius of 1.4 cm. when inoculated at the periphery with a small piece of the complementary mycelium. During the five weeks following this inoculation, the original haploid mat grew 2.1 cm. in radius on the side away from the inoculation, showing that the haploid mycelium continued to grow at its customary rate. The diploid mycelium, during the same five weeks, extended radially to a distance of 5.5 cm., gradually encompassing the haploid. At the time the photograph was taken, the haploid mycelium retained its typical haploid appearance, was powdery with abundant oidia, and no clamp connections could be found anywhere upon that area.

Haploid mycelia originating from basidiospores of the same pileus showed great variation in the rate of growth. Some were very slow, attaining a diameter of only 2 cm. in two weeks at 20° C., while others grew to 7 cm. in diameter under the same conditions during the same period.

In all the haploid mycelia of the three species of *Collybia* studied, oidia were produced, generally in large quantities, formed by the breaking-up of the mycelium into individual cells which vary in length. In the slower growing haploid mycelia, practically the whole mycelial mass breaks up into oidia, while in

the faster growing haploids, the oidia make up a much smaller proportion of the mat. In both cases, however, the oidia are responsible for the opaque powdery appearance of the mycelium. The writer has never seen oidia on the diploid mycelia of the *Collybia* species studied.

Diploid mycelia of the three species of *Collybia* are easily distinguishable from each other. When the diploid mycelium of *C. tuberosa* is seven to ten days old, white beaks or small horns push out from the oldest part of the mat. These are accompanied by the watery exudate so characteristic of sclerotia formation (PLATE 36, FIG. 1). These horns are sclerotia, but under the conditions of culture they do not become hard and horny as in nature, and only a few take on the dark reddish brown color (PLATE 36, FIG. 8). The dark colored sclerotia do not produce fruit bodies as quickly as those which remain light colored. In the field, the sclerotia are clearly delimited from the stipe. In culture the sclerotium and stipe are not distinct from each other. Some of the sclerotia in culture show a rudimentary pileus at a very early age, without a well differentiated stipe (PLATE 36, FIG. 8). Later the sclerotium elongates and the pileus expands (PLATE 36, FIG. 7). These sclerotial "horns" or "beaks" are very sensitive to light, bending directly toward it (PLATE 36, FIG. 8). When the sclerotia-like bodies are injured or cut, there is a marked tendency to proliferate.

The diploid mycelium of *C. cirrata* differs from those of the other two species in having no sclerotium. It is a whitish mouldy growth with no special feature of identification (PLATE 36, FIG. 3).

C. cirrata var. *Cookei* is identified by its large irregular yellow sclerotia which soon form on the diploid mycelium. A large sclerotium or group of sclerotia develops in the oldest part of the mat first (PLATE 36, FIG. 2), and later as the mycelium extends, a ring of sclerotia may form at some distance out from the center. Large drops of watery exudate collect around the developing sclerotia (PLATE 36, FIG. 6 AND 10).

Sclerotia are also produced from haploid mycelia of *C. cirrata* var. *Cookei*, but in *C. tuberosa* no sclerotia have been seen on the haploid mycelia, although there are frequently drops of water to

be seen in the central part of the haploid mycelia, suggesting an attempt to form sclerotia.

EFFECT OF TEMPERATURE ON GROWTH. A study was made with regard to the effect of temperature on the rate of growth of diploid mycelia of four species of *Marasmius* and three of *Collybia*. These were grown in petri dishes in triplicate, using Kauffman's nutrient agar. The cultures were grown at nine different temperatures. The results are given in the following table:

TABLE I

AVERAGE GROWTH IN MM. OF DIPLOID MYCELIA OF SPECIES OF *Marasmius* AND *Collybia* AT END OF TENTH DAY AT VARIOUS TEMPERATURES

Species	Temperatures in Degrees Centigrade								
	3	5	10	14	20	25	29	35	40
M. alliatus	0	0	13	18	37	38	42	18	0
M. capillaris	0	1	13	34	48	52	0	0	0
M. elongatipes	2	6	35	43	58	41	7	0	0
M. epiphyllus	8	14	36	48	65	46	1	0	0
C. cirrata	2	4	0	7	23	19	0	0	0
C. cirrata var. *Cookei*	5	14	17	23	39	21	0	0	0
C. tuberosa	4	8	11	16	33	15	0	0	0

From this table it is evident that there is a decided difference in the reaction of the species to temperature. The lower temperatures inhibited the growth of *M. alliatus* and *M. capillaris* much more than *M. elongatipes*, *M. epiphyllus* and the three species of *Collybia*. *M. alliatus* was outstanding for its ability to develop at the higher temperatures. For *M. elongatipes*, *M. epiphyllus*, *C. cirrata*, *C. cirrata* var. *Cookei* and *C. tuberosa*, the optimum temperature lies at about 20° C., whereas for *M. capillaris* it is probably between 20° C. and 25° C. The sudden cessation of growth between 25° C. and 29° C. leads one to believe that perhaps the optimum lies somewhat below 25° C. *M. alliatus* has a rather high optimum temperature, probably somewhere between 25° C. and 29° C., judging from the rapid decrease in the amount of growth at temperatures above 29° C. Compared at their respective optimum temperatures, *M. epiphyllus* was the most rapidly growing species.

The rate of growth of each of the species of *Collybia* appears to be quite distinct, with *C. cirrata* var. *Cookei* growing most

rapidly of the three, while *C. cirrata* grew most slowly. For some reason the three cultures of *C. cirrata* at 10° C. failed to grow, otherwise all three of the species of *Collybia* show a steady increase in growth as the temperature increases to the optimum 20° C.

SEXUALITY. The sexual reactions of the fungi were studied in the usual way, *i.e.*, by pairing monosporous mycelia from basidiospores of a single pileus in all possible combinations. The results will be discussed by species.

1. *Marasmius elongatipes* Peck. When monosporous cultures from a single pileus of this species are paired in all possible combinations, three types of reactions are distinguishable. First, a complete mingling of two mycelia (*a*) when of the same sex, originally derived from the same basidiospore, or, in other words, where the genetic constitution is exactly the same in each mycelium; (*b*) when two sexually compatible mycelia are paired.

Second, a tendency for the two mycelia not to mingle but to remain separated from each other by a line of demarcation of greater or less degree. This reaction appeared between any two incompatible mycelia of different sexual groups (PLATE 35, FIG. 2, 3 AND 5), and it was also found to occur between two mycelia of the same sex which originated from different basidiospores (PLATE 35, FIG. 4). Sometimes the line of demarcation appears as a clear zone, and sometimes as a heaping up of mycelium, as in the last mentioned illustration. The writer has not been able to detect any correlation between this "aversion" and any particular sexual factor.

The third type of reaction is a general diploidization of the two mycelia when they are of complementary sexual constitution (PLATE 35, FIG. 6). Here there is a complete mingling, as mentioned above. A diploid mycelium in this species may be brown and felty or white and fluffy. This apparently depends upon the nature of the haploids which unite. Some of the haploid mycelia are brown, and some are pure white. A study of this color factor has shown that wherever two incompatible mycelia are paired, one brown and one white, there is no mingling of the two. Also, wherever a white haploid mycelium is paired with a brown haploid of complementary sex, the resulting diploid mycelium is brown. If two white haploid mycelia of opposite sex are

paired, the resulting diploid mycelium is white. Brown and white mycelia are found in each of the sexual groups. This offers interesting possibilities for a genetical study.

Marasmius elongatipes Peck, collection 2. This collection was made in the vicinity of Ann Arbor, Michigan, about Oct. 1, 1931, by E. B. and E. E. Mains (*31–897*). Monosporous mycelia were obtained (using fresh material) from two different pilei. In the first case 15 spores were isolated, and in the second case 11 were obtained. Within each group pairings were made in every possible combination, with results as given in tables II and III.

TABLE II

Marasmius elongatipes PECK. RESULTS OF PAIRING 15 MONOSPOROUS MYCELIA ISOLATED FROM PILEUS 1

		AB						Ab		aB				ab		
		1	6	7	8	14	15	5	17	12	16	18	19	3	4	9
AB	1	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	6	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	7	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	8	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	14	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	15	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Ab	5	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−
	17	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−
aB	12	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
	16	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
	18	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
	19	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
ab	3	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
	4	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
	9	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−

+, clamp connections formed
−, no clamp connections formed

The monosporous mycelia of pileus 2 were tested against monosporous mycelia 1, 5, 12 and 3 from pileus 1, in order to assign them to their proper sexual group. The species is clearly tetrapolar in the arrangement of its sexual factors. Of the total 26 haploid mycelia, seven had the genetic constitution AB, eight were Ab, five were aB and six were ab.

Marasmius elongatipes Peck, collection 32. This group of fruit bodies was collected in Saginaw Forest, Ann Arbor, Michigan, on Nov. 20, 1931, by E. B. and E. E. Mains (*31–898*).

TABLE III

Marasmius elongatipes PECK. RESULTS OF PAIRING 11 MONOSPOROUS MYCELIA ISOLATED FROM PILEUS 2

		AB	Ab					aB	ab		
		33	21	25	27	31	34	35	28	20	23	32
AB	33	−	−	−	−	−	−	−	−	+	+	+
Ab	21	−	−	−	−	−	−	−	+	−	−	−
	25	−	−	−	−	−	−	−	+	−	−	−
	27	−	−	−	−	−	−	−	+	−	−	−
	31	−	−	−	−	−	−	−	+	−	−	−
	34	−	−	−	−	−	−	−	+	−	−	−
	35	−	−	−	−	−	−	−	+	−	−	−
aB	28	−	+	+	+	+	+	+	−	−	−	−
ab	20	+	−	−	−	−	−	−	−	−	−	−
	23	+	−	−	−	−	−	−	−	−	−	−
	32	+	−	−	−	−	−	−	−	−	−	−

Fifteen spores were isolated from one of the pilei while fresh, and the monosporous mycelia obtained from them were paired in all possible combinations, with results as given in table IV.

This table also shows the species to be tetrapolar. The twenty-six monosporous mycelia obtained from collection 2 were paired with four tester mycelia from collection 32, namely mycelia 7, 15, 26 and 2. Of the 104 pairings, 103 showed clamp connections. The other culture became contaminated. These results compare well with similar data submitted by Hanna (**10**) for *Coprinus lagopus* where complete interfertility was the rule in such crossings.

Pennington (**19**) gives the size of the basidiospores in this species as 7–8 × 3.5 μ. Collection 63, made by the writer at Arms Lake, Michigan, Oct. 27, 1932, had spores which measured 8.9–9.9 (10.5) μ × 3.3–3.6 μ when selected at random. The basidia bore four spores, so the larger spore size cannot be attributed to the number of spores produced as has been found by Smith (**21**) for two-spored forms of *Mycena*.

TABLE IV

Marasmius elongatipes PECK. RESULTS OF PAIRING 15 MONOSPOROUS MYCELIA ISOLATED FROM ONE PILEUS OF COLLECTION 32

		AB					Ab					aB			ab	
		1	3	7	14	19	4	13	15	17	20	18	25	26	2	12
AB	1	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	7	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	19	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
Ab	4	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−
	13	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−
	15	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−
	17	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−
	20	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−
aB	18	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−
	25	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−
	26	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−
ab	2	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	12	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−

This was such a striking deviation from the published size that it was considered desirable to pair monosporous mycelia of the long-spored form with monosporous mycelia of collections 2 and 32, both of which had spores agreeing very well with the size given by Pennington. Unfortunately, only one of the original monosporous mycelia from collection 63 survived in culture, so that it became necessary to hunt the long-spored form anew in the fall of 1933. As collection after collection was brought in and measured, it appeared that there were all variations in basidiospore size ranging from the size given by Pennington up to that found by the writer in the collection which came from Arms Lake. In order to study this variation, several collections of *M. elongatipes* were made in close proximity to each other at Cascade Glen, near Ann Arbor, Michigan, on Oct. 23, 1933. Mature pilei were selected from these collections and allowed to deposit their spores in sterile capsules. The pilei were selected from groups growing within a few feet or at most a few yards of each other, and were labelled A, B, C, D and E respectively. Twenty spores

were selected at random from a spore print from each of the pilei, with results as given in table V.

TABLE V

Marasmius elongatipes PECK. SHOWING VARIATION IN LENGTH OF BASIDIOSPORES FROM FIVE COLLECTIONS OF THE SAME SPECIES

Pileus	Range of length of spores	Average length
A	7.6– 9.0	8.3
B	7.6– 9.6	8.6
C	5.6– 8.5	7.0
D	7.4–11.2	9.3
E	7.6–10.1	8.8

From these figures it is seen that the two populations C and D are extremes, each occupying a distinct range which only slightly overlap each other. The other populations occupy a range slightly intermediate between those of C and D.

The sexual reaction of the original long-spored form (collection 63) from Arms Lake was determined by mating 8 monosporous mycelia in the usual way. The results of these pairings are given in table VI.

TABLE VI

Marasmius elongatipes PECK. RESULTS OF PAIRING 8 MONOSPOROUS MYCELIA OF THE LONG-SPORED FORM, COLLECTION 63

		AB		Ab				aB	
		6	9	1	3	13	14	4	7
AB	6	—	—	—	—	—	—	—	—
	9	—	—	—	—	—	—	—	—
Ab	1	—	—	—	—	—	—	+	+
	3	—	—	—	—	—	—	+	+
	13	—	—	—	—	—	—	+	+
	14	—	—	—	—	—	—	+	+
aB	4	—	—	+	+	+	+	—	—
	7	—	—	+	+	+	+	—	—

The long-spored form exhibits the tetrapolar arrangement of sexual factors as does the short-spored form, although in the eight spores isolated in this instance, only three of the sexual groups are represented.

Unfortunately only one of these monosporous mycelia survived, but it was paired with eight monosporous mycelia from each of the short-spored collections 2 and 32, and showed complete fertility with all of them. The eight monosporous mycelia from collections 2 and 32 contained two representatives of each of the four sexual groups in each case. See tables VII and VIII.

TABLE VII

Marasmius elongatipes PECK. RESULTS OF PAIRING HAPLOID MYCELIUM 4 (GENETIC CONSTITUTION aB) OF THE LONG-SPORED COLLECTION 63 WITH 8 HAPLOID MYCELIA OF SHORT-SPORED COLLECTION 2

	AB	Ab	aB	ab				
2 / 63	1	6	5	21	12	16	3	4
4	+	+	+	+	+	+	+	+

TABLE VIII

Marasmius elongatipes PECK. RESULTS OF PAIRING HAPLOID MYCELIUM 4 (GENETIC CONSTITUTION aB) OF THE LONG-SPORED COLLECTION 63 WITH 8 HAPLOID MYCELIA OF SHORT-SPORED COLLECTION 32

	AB	Ab	aB	ab				
32 / 63	1	3	4	13	18	25	2	12
4	+	+	+	+	+	+	+	+

Complete fertility was also found when nine monosporous mycelia of known sexual reaction, from the long-spored population D, were paired with four haploid mycelia of collection 2 and four of collection 32. The results of these pairings are given in tables IX and X.

From these data it is seen that the long-spored forms behave like the other collections of this species. The description of the species should be amended to read "basidiospores very variable as to length, 6.6–11, averaging 7–9.7 by 3.3–3.6 in width."

As has been pointed out above, the situation in this species is comparable to that in *Coprinus lagopus*, where Hanna (**10**) discovered that between different strains of the fungus there is complete fertility.

2. *Collybia cirrata* FRIES. The material for this study was collected by the writer in Saginaw Forest, Ann Arbor, Michigan, Oct. 1, 1932 (*3220*). In this region it was quite abundant, grow-

TABLE IX

Marasmius elongatipes PECK. RESULTS OF PAIRING 9 MONOSPOROUS MYCELIA OF LONG-SPORED POPULATION D WITH 4 MONOSPOROUS MYCELIA OF SHORT-SPORED COLLECTION 2

TABLE X

Marasmius elongatipes PECK. RESULTS OF PAIRING 9 MONOSPOROUS MYCELIA OF LONG-SPORED POPULATION D WITH 4 MONOSPOROUS MYCELIA OF SHORT-SPORED COLLECTION 32

TABLE IX

		AB		Ab	
	D 2	1	6	5	21
AB	1	+	+	+	+
	5	+	+	+	+
	6	+	+	+	+
	7	+	+	+	+
	9	+	+	+	+
Ab	3	+	+	+	+
	8	+	+	+	+
ab	2	+	+	+	+
	4	+	+	+	+

TABLE X

		AB		Ab	
	D 32	1	3	4	13
AB	1	+	+	+	+
	5	+	+	+	+
	6	+	+	+	+
	7	+	+	+	+
	9	+	+	+	+
Ab	3	+	+	+	+
	8	+	+	+	+
ab	2	+	+	+	+
	4	+	+	+	+

ing amongst pine needles on the withered remains of *Boletus luteus*. This species of *Collybia* does not develop sclerotia. The base of the stipe is provided with very characteristic strigose hairs, and is distinctly rooting.

At first, only eight monosporous mycelia were obtained from this species, and they were paired together to ascertain the sexual conditions. The results of these pairings are given in table XI.

TABLE XI

Collybia cirrata FRIES. RESULTS OBTAINED BY PAIRING 8 MONOSPOROUS MYCELIA IN ALL POSSIBLE COMBINATIONS

		AB			Ab			ab	
		1	4	7	2	6	8	3	5
AB	1	−	−	−	−	−	−	+	+
	4	−	−	−	−	−	−	+	+
	7	−	−	−	−	−	−	+	+
Ab	2	−	−	−	−	−	−	−	−
	6	−	−	−	−	−	−	−	−
	8	−	−	−	−	−	−	−	−
ab	3	+	+	+	−	−	−	−	−
	5	+	+	+	−	−	−	−	−

Since the reaction showed a clear case of tetrapolarity in which the sexual group aB was not represented, 23 additional spores were isolated and tested against three of these mycelia showing different sexual reaction. These results are given in table XII.

TABLE XII

Collybia cirrata FRIES. DETERMINATION OF SEX OF 23 ADDITIONAL MONOSPOROUS MYCELIA

		AB					Ab		aB						ab										
		9	13	27	33	42	17	40	11	18	21	23	29	31	20	22	25	26	37	38	39	43	44	45
AB	1	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
Ab	2	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
ab	3	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Combining the two groups of monosporous mycelia isolated, it was found that of the total 31 mycelia, 8 had the genetic constitution AB, 5 were Ab, 6 were aB and 12 were ab.

3. *Collybia cirrata* Fries var. *Cookei* Bres. This form was collected by the writer upon humus in the woods near Duck Lake, Pinckney, Michigan, Sept. 24, 1932 (*321*). It has also been collected by A. H. Smith, who found it in the vicinity of Ann Arbor, Michigan, Oct. 6, 1932, growing upon soil, and by E. B. Mains, who found some on soil and some on agaric material at Lakeland, Michigan, Oct. 15, 1932 (*32–879*). In all cases the form is readily identified by the presence of a yellow nodular sclerotium at the base of the stipe. Seventeen monosporous mycelia were obtained and paired with each other as illustrated in table XIII.

In this case the inhibition factor or factors do not appear to be connected with any of the factors for sex determination, and lines of demarcation were frequently found between mycelia of the same sex but originating from different spores.

The proportions of the different sexual groups being so unequal in this experiment, 13 more spores were isolated and their mycelia tested as to sex. The results are given in table XIV.

Thus, in the 30 monosporous mycelia isolated altogether for this species, 5 had the constitution AB, 10 were Ab, 9 were aB and 6 were ab, giving another example of tetrapolarity.

Three types of reactions were observed here as in the case of *Marasmius elongatipes*, when haploid mycelia were paired. The first type of reaction, a mingling of the two mycelia, is illustrated on plate 36, figure 5. The second type, where a line of demarcation is formed between two mycelia, is illustrated on plate 36,

TABLE XIII

Collybia cirrata FRIES VAR. *Cookei* BRES. RESULTS OF PAIRING 17 MONOSPOROUS MYCELIA IN ALL POSSIBLE COMBINATIONS

		AB		Ab								aB					ab
		2	10	6	17	19	20	22	23	24	25	26	8	9	12	15	16	3
AB	2	—	·/—	·/—	·/—	·/—	·/—	·/—	·/—	·/—	—	·/—	·/—	·/—	·/—	·/—	·/—	+
	10	·/—	—	·/—	—	·/—	·/—	·/—	—	—	—	—	·/—	—	—	—	·/—	+
Ab	6	·/—	·/—	—	—	—	—	—	·/—	·/—	—	—	+	+	+	+	+	—
	17	·/—	—	—	—	c	·/—	—	—	—	—	—	+	+	+	+	+	·/—
	19	·/—	·/—	—	c	—	—	—	—	—	—	—	+	+	+	+	+	—
	20	·/—	·/—	—	·/—	—	—	—	—	—	—	—	+	+	+	+	+	—
	22	·/—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	—
	23	·/—	—	·/—	—	—	—	—	—	—	—	—	+	+	+	+	+	—
	24	·/—	—	·/—	—	—	—	—	—	—	—	—	+	+	+	+	+	—
	25	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	·/—
	26	·/—	—	—	—	—	—	—	—	—	—	—	+	+	+	c	+	·/—
aB	8	·/—	·/—	+	+	+	+	+	+	+	+	+	—	—	·/—	·/—	—	—
	9	·/—	—	+	+	+	+	+	+	+	+	+	—	—	—	·/—	—	·/—
	12	·/—	—	+	+	+	+	+	+	+	+	+	·/—	—	—	·/—	·/—	·/—
	15	·/—	—	+	+	+	+	+	+	+	+	c	·/—	·/—	·/—	—	·/—	·/—
	16	·/—	·/—	+	+	+	+	+	+	+	+	+	—	—	·/—	·/—	—	·/—
ab	3	+	+	—	·/—	—	—	—	—	—	·/—	·/—	—	·/—	·/—	·/—	·/—	—

c, contamination prevented observation
·, inhibition, *i.e.*, distinct line between mycelia
+, clamp connections present
—, clamp connections not formed

TABLE XIV

Collybia cirrata FRIES VAR. *Cookei* BRES. DETERMINATION OF THE SEX OF 13 ADDITIONAL MONOSPOROUS MYCELIA

		AB			Ab	aB				ab				
		37	40	41	33	27	30	36	42	29	35	43	44	45
AB	2	—	—	—	—	—	—	—	—	+	+	+	+	+
Ab	6	—	—	—	—	+	+	+	+	—	—	—	—	—
aB	8	—	—	—	+	—	—	—	—	—	—	—	—	—
ab	3	+	+	+	—	—	—	—	—	—	—	—	—	—

figure 6, while the third type, the diploidization reaction, is illustrated in figure 4 of the same plate, and presents a very different picture to that found in *Marasmius elongatipes*.

4. *Collybia tuberosa* Fries. Specimens of this species were collected in the summer of 1932 on decayed agaric material in a swamp at Rock River, Michigan, by E. B. and E. E. Mains (*32–184*). That fall, it was also collected by the writer at Oscoda, Michigan (*3266*), where it was growing upon decayed agaric material and also amongst pine needles where decayed agaric

TABLE XV

Collybia tuberosa FRIES. RESULTS OF PAIRING 17 MONOSPOROUS MYCELIA IN ALL POSSIBLE COMBINATIONS

		AB						Ab		aB	ab						
		1	4	9	13	15	22	5	18	27	10	16	21	23	25	28	29	31
AB	1	−	−	−	−	−	−	−	−	−	+	+	+	c	+	†−	+	c
	4	−	c	−	*−	−	−	−	−	−	+	c	+	+	+	+	+	+
	9	−	−	−	−	−	−	−	−	−	+	+	†−	+	+	+	+	+
	13	−	*−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	15	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	22	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
Ab	5	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
	18	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
aB	27	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−
ab	10	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	16	+	c	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	21	+	+	†−	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	23	c	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	25	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	28	†−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	29	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	31	c	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−

* Clamp connections began to form but did not completely fuse. Appeared as small curved branches only occasionally bridging over septum to touch neighboring cell, presumably "Pseudoschnallen" as described by Brunswik (**5**).

† These exceptions were very carefully checked, but no clamp connections were found. The apparent sterility in these cases can not be due to physiological immaturity of the mycelia, as they form clamp connections with other complementary mycelia. It may be due to an insufficient difference in the sexual "realisateurs" of Wettstein as Vandendries (**23**) suggests in similar instances.

material was not evident. The most characteristic feature of this species is its small dark reddish brown sclerotium, which is shaped somewhat like an elongated and rather shrivelled apple seed. It appeared to be growing upon withered pilei of *Russula*, but it was not possible to determine whether it is limited to this genus.

The results of pairing 17 monosporous mycelia of this species are given in table XV.

ATTEMPTS TO PRODUCE INTERSPECIFIC CROSSES. Since the three forms of *Collybia* used in this study appear to be closely related to each other, it seemed desirable to use the material in an attempt to obtain crosses between them. Tables XVI, XVII and XVIII embody the results of such experiments.

TABLE XVI

THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS OF *C. tuberosa* WITH THOSE OF *C. cirrata*

			C. tuberosa						
			AB		Ab		aB	ab	
			13	15	5	18	27	10	25
C. cirrata	AB	33	—	—	—	—	—	—	—
		42	—	—	—	—	—	—	—
	Ab	2	—	—	—	—	—	—	—
		6	—	—	—	—	—	—	—
	aB	21	—	—	—	—	—	—	—
		23	—	—	—	—	—	—	—
	ab	39	—	—	—	—	—	—	—
		44	—	—	—	—	—	c	—

—, no clamp connections formed; c, culture contaminated

TABLE XVII

THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS OF *C. tuberosa* WITH THOSE OF *C. cirrata* VAR. *Cookei*

			C. tuberosa						
			AB		Ab		aB	ab	
			13	15	5	18	27	10	25
C. cirrata var. Cookei	AB	37	—	—	—	—	—	—	—
		40	—	—	—	—	—	—	—
	Ab	19	c	—	—	—	c	—	—
		22	—	—	—	—	—	—	—
	aB	12	—	—	—	—	—	—	—
		30	—	—	—	—	—	—	—
	ab	35	—	—	—	—	—	—	—
		43	—	—	—	—	—	—	—

—, no clamp connections formed; c, culture contaminated

TABLE XVIII

THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS OF *C. cirrata* WITH THOSE OF *C. cirrata* VAR. *Cookei*

			C. cirrata							
			AB		Ab		aB		ab	
			1	4	2	6	9	18	3	5
C. cirrata var. Cookei	AB	2	—	—	—	—	—	—	—	—
		10	—	—	—	—	—	—	—	—
	Ab	19	—	—	—	—	—	—	—	—
		17	—	—	—	—	—	—	—	—
	aB	8	—	—	—	C	—	—	—	—
		9	—	—	—	—	—	—	—	—
	ab	45	—	—	—	—	—	—	—	—
		44	—	—	—	—	—	—	—	—

The results of the foregoing attempts to produce interspecific crosses have all been negative. Complete sterility appears to exist between these forms. Kniep (**14**) reported an experiment which he performed in connection with his studies upon the sex of *Collybia cirrata* Fries which is interesting in this regard. He obtained from two localities in Brandenburg (Havelberg and Finkenkrug) what he thought to be two collections of *C. cirrata*. He paired them, and found them to be completely intersterile. His statement (**14**, p. 414) reads as follows: "Die kleinen charakteristischen, in grossen Mengen auf verrotteten Pilzen auftretenden Fruchtkörper beiderlei Herkunft stimmten völlig miteinander überein, ebenso die Sporen und die daraus kultivierten Myzelien, die durch einen äusserst auffallended Erdgeruch ausgezeichnet sind. Der einzige Unterschied war, dass der Pilz von Finkenkrug sehr stark zur Sklerotienbildung neigte, der andere nicht. Die Haplonten beider Pilze waren gegeneinander völlig steril."

It is evident that he was dealing with *Collybia cirrata* Fries and *C. cirrata* Fries var. *Cookei* Bres.

METHODS OF DIPLOIDIZATION. The species of fungi studied in this investigation present two distinct types of diploidization. One type is found in *Marasmius elongatipes*, the other type is exhibited by the three species of *Collybia*. In *Marasmius elongatipes* diploidization occurs in the same manner as that described by Buller (**6**) for *Coprinus lagopus*. In this type, when two haploid mycelia of complementary sexual constitution

are paired, the diploid condition arises first at the line of contact of the two mycelia and spreads from there through both of the original haploid mycelia, progressing more rapidly in the younger parts of these mats. It is very difficult to see any macroscopic differences between haploid and diploid mycelia of *M. elongatipes*, if one leaves out of consideration the difference in the rate of growth of haploid and diploid mycelia. The method recommended by Buller (6) for studying diploidization, namely that of inoculating a large mat of a haploid mycelium with a small piece of a complementary mycelium at the periphery, brought out clearly the difference in the two kinds of diploidization. In plate 36, figure 6, a large mat of haploid mycelium 7 of *Marasmius elongatipes*, with the genetic constitution AB, was inoculated at a point on its margin with a small piece of haploid mycelium 2, whose genetic constitution was ab. At the time the photograph was taken, two weeks later, the culture was examined and it was seen that the diploid condition had spread through much of the haploid mycelium 7. The extent of the diploid condition, as determined by the presence of clamp connections, is shown by the letter d, while the areas in which clamp connections were not found are marked h, presuming that such areas are still haploid.

The three species of *Collybia* showed a very different phenomenon from that of *Marasmius elongatipes*. In this case, when two complementary haploid mycelia were paired, the diploid mycelium formed along the line of contact, but the mycelia originally haploid remained so. The diploid mycelium grows so much faster than the haploid that it soon grows out and surrounds the latter. It is easy to tell even macroscopically which is haploid and which is diploid mycelium (PLATE 36, FIG. 4). Large haploid mycelia were inoculated with a small amount of their complementary mycelium placed at a point on their margin, and the manner of diploidization is shown on plate 36, figure 10. The haploid mycelium remains haploid and retains the opaque powdery appearance characteristic of the haploid mycelia of this group, while the diploid mycelium forms as a fan, gradually surrounding the haploid, and easily distinguishable from it by the possession of rather coarse radiating hyphae which are quite conspicuous. This might be regarded as a sort of "limited diploidization," for

there is certainly no progressive transformation of haploid into diploid mycelium in this case. This "limited diploidization" may be similar to that which Vandendries (**26**) has recently reported in *Trametes suaveolens*.

STUDIES CONCERNING MYCELIAL FUSIONS. Davidson, Dowding and Buller (**7**) made careful observations on the formation of fusions between different mycelia in their study upon dermatophytes. They found that fusions would occur only between mycelia of the same species, and were enabled thereby to identify species of dermatophytes much more quickly than before. Vandendries (**28**) has found that this is true also for basidiomycetes, and that all mycelia of one and the same species are capable of forming mycelial fusions with each other, even though they may be sexually incompatible haploids. It seemed advisable to subject the three forms of *Collybia* concerned in this study to such a test. Diploid mycelia of *C. cirrata* were paired with diploid mycelia of *C. cirrata* var. *Cookei* on an agar film in Van Tieghem cells as described by Davidson, Dowding and Buller (**7**). Similar pairings were made of diploid mycelia of *C. cirrata* and *C. tuberosa*, and of *C. cirrata* var. *Cookei* and *C. tuberosa*. Pairings were also made between two diploid mycelia of the same species in each case, *e.g.*, *C. tuberosa* with *C. tuberosa*. There were numerous fusions observed between mycelia of the same species, and between different branches of the same mycelium, but no mycelial fusions were noted in which two mycelia of different species were involved. Neither were there any fusions between the diploid mycelia of *C. cirrata* and *C. cirrata* var. *Cookei*. In this instance, moreover, it was observed that *C. cirrata* var. *Cookei* seemed to be adversely affected when its hyphal tips penetrated into the edge of the area covered by the mycelium of *C. cirrata*, for its tip branches became distorted and gnarled, and produced several unnaturally short and stunted branchlets at the end of the hyphae. No such reaction occurred between *C. cirrata* and *C. tuberosa*, or between *C. cirrata* var. *Cookei* and *C. tuberosa*.

SPOROPHORE PRODUCTION UPON ARTIFICIAL MEDIA. *Marasmius elongatipes* Peck. (PLATE 35, FIG. 1) fruited readily upon several media including malt agar, medium 5, medium 10, Kauffman's nutrient agar, and modifications of Poole's medium

and Etter's medium. On Kauffman's agar the fruit bodies were not normal, and did not produce spores. On all the other media the sporophores were practically normal, except that the stipe in some cases was thicker than it is in nature. From three of the compatible matings between collections 2 and 32 of this species, eight small sporophores were produced in the petri dishes.

Marasmius capillaris Morg. fruited upon medium 5, while *M. epiphyllus* Fries fruited upon medium 5 and medium 10.

Collybia cirrata, *C. cirrata* var. *Cookei* and *C. tuberosa* all fruited on various media which contained as a base the dried pilei of *Boletus luteus*. Although *C. tuberosa* is reported as usually growing upon remains of species of *Russula*, and *C. cirrata* var. *Cookei* generally on soil, it was interesting to see them thrive on *Boletus luteus*, the substratum on which *C. cirrata* grows in this region.

An attempt was made to grow the species of *Marasmius* upon the *Boletus* medium, but not the slightest trace of growth of these species could be obtained on this material.

None of the above-mentioned species produced fruit bodies when grown in the dark, nor have sporophores been produced by any of the haploid cultures up to the present time.

LONGEVITY AND ABILITY OF PILEI TO REVIVE. From time to time during the course of this study, attempts were made to obtain spore deposits from revived pilei of various species of *Marasmius* and *Collybia*. *Marasmius elongatipes*, *M. delectans*, *M. alliatus*, *M. urens* and *M. rotula* all gave negative results. They regained their normal shape and texture, but deposited no spores. *Collybia cirrata* var. *Cookei* and *C. tuberosa*, however, gave better results. *C. cirrata* var. *Cookei* when revived after 51 days of storage in a dry condition gave a good spore deposit when suspended over an agar plate, and *C. tuberosa*, after 86 days in a dried state, also yielded a good spore print. Moreover, the spores in both instances were viable. In each case, two out of four revived pilei deposited their spores readily.

DISCUSSION. Two different types of diploidization have been noted in these studies. Buller (**5**), in his work upon *Coprinus lagopus*, found that when two monosporous mycelia of complementary sexual constitution were paired, the diploid condition

formed first at the line of contact of the two mycelia and subsequently spread throughout both mycelia, advancing more rapidly through the young hyphae than through the older. This he believed due to the passage of rapidly-generated daughter nuclei of the one mycelium travelling through the other mycelium and becoming associated with the nuclei of the mycelium through which they were passing to form pairs of nuclei which thereafter would divide conjugately. As has been shown, *Marasmius elongatipes* behaves very much the same as *Coprinus lagopus*.

In the three species of *Collybia* studied, an entirely different kind of diploidization prevails. Here there is no spread of the diploid condition through or across the paired haploids. The diploid cells arise merely at the line of contact, and from them diploid hyphae develop and spread out in fans from between the two haploid mycelia. On account of its greater rapidity of growth, the diploid mycelium grows around the two haploids, but in doing so, it encroaches little, if any, upon them. In the culture shown on plate 36, figure 10, it can be seen that the haploid mycelium continued to grow at its usual rate even after the diploid mycelium had begun to develop.

While *Collybia cirrata*, *C. cirrata* var. *Cookei* and *C. tuberosa* all present this "limited diploidization," it is interesting to note that H. J. Brodie (**4**) has reported that *C. velutipes* Fries has a type of diploidization resembling that of *Coprinus lagopus*.

Several cases have been reported where the diploid mycelium appears to be limited to a narrow region along the line of contact. Vandendries (**26**) has made mention of this condition in *Trametes suaveolens*. The same author in collaboration with H. J. Brodie has recorded it also in *Polystictus versicolor*, and Smith and Brodie (**22**) in an article on *Pholiota polychroa* (Berk.) Smith & Brodie have pointed out the same fact. In all of these cases, however, there appears to be considerable growth of the haploids before there is any sign of diploid mycelium forming, and when it does appear, the diploid mycelium does not seem to have the greatly accelerated rate of growth that it has in the case of the *Collybia* species. In all of these cases also the authors report that the diploid mycelium grows across or over the haploid, the diploid and haploid mycelia mingling until finally there is great

difficulty in finding a trace of the original haploid mycelia. This is quite different from the situation in the three species of *Collybia* described in the present paper, although in all these cases the diploidization might be described as strictly "limited."

The variations in reactions between different haploid mycelia of a single species when paired together present perplexing questions. "Lines of demarcation," "inhibition," "Hemmungsreaktion," "aversion," "repulsion" and "barrage" are terms which have been used by different investigators to denote various types of incompatibility.

Some investigators have found in the results of their experiments very clear cut reactions. Vandendries (**25**) found that "barrage" was dependent on certain combinations of Mendelian factors, and traced a distinct correlation between the occurrence of "barrage" and the common possession of the a or a′ factor by the two paired mycelia. He maintains (**27**) that this "barrage sexuel" cannot exist in bipolar species, and must not be confused with the phenomena of demarcation which do not behave in a Mendelian fashion.

In *Marasmius elongatipes* it is evident that there is something in the nature of an inhibition or demarcation factor which is not associated with the sexual factors. In fact, demarcation between mycelia in this species is more prevalent than mingling of mycelia, for complete mingling only occurred when diploidization took place or when the paired mycelia had originated from the same spore. Even mycelia of the same sexual constitution refused to mingle if they had arisen from different spores. Miss Mounce (**17**) found in her studies on *Fomes pinicola* that lines of demarcation were very general between monosporous (and even between diploid) mycelia, and that mingling occurred only between "closely related" mycelia. Oort (**18**) reported similar conditions in *Coprinus fimetarius* when he distinguished between "OO-Kombinationen" (mycelia from the same basidiospore) and "O-Kombinationen" (mycelia of the same sex but from different basidiospores), and reported that in the latter combinations he sometimes found a weak repulsion.

Marasmius elongatipes presents an unusual condition in regard to the variation in size and shape of basidiospores. The spore

size is commonly given as 7–8 × 3.5 μ (**19**) for this species. However, in normally deposited spore prints some spores have been found only 5.6 μ in length, while others measured as much as 11.2 μ in length. The width varied very slightly, ranging from 3.3 μ to 3.6 μ; consequently the variation in length involved a great diversity of spore forms as well as spore dimensions. The range of size in the short-spored group slightly overlapped the range of the large-spored group, while other populations were intermediate. When mated, the sexual strains of the long-spored group were completely interfertile with those of the short-spored group, showing that they are races of the same species.

In studying the relationship of *Collybia cirrata* Fries var. *Cookei* Bres. to *C. cirrata* Fries and *C. tuberosa* Fries, all attempts to produce interspecific hybrids yielded only negative results. Microscopic examination showed that there were no mycelial fusions between these species when grown together. The diploid mycelium of *C. cirrata* var. *Cookei* became greatly branched and stunted at the tips of the hyphae when grown along with *C. cirrata*. This was the only case observed where one mycelium had a visible effect on the other. The complete absence of mycelial fusions between the three forms when paired with each other, and the fact that the production of sclerotia was a consistent feature of the diploid mycelia of *C. tuberosa* and *C. cirrata* var. *Cookei*, while sclerotia were never developed in *C. cirrata*, furnish evidence that the agaric hitherto recognized as *Collybia cirrata* Fries var. *Cookei* Bres.[2] is in reality a specific entity.

All three species of *Collybia* studied showed the same type of oidia on the haploid mycelia. No oidia were observed on diploid mycelia of these species of *Collybia*. On *Marasmius elongatipes* no oidia were produced either on the haploid or on the diploid mycelia.

The power of revival has long been considered a diagnostic character of the genus *Marasmius*. When a dried and shrivelled specimen of *Marasmius* is placed for a time in a moist chamber, there will be a marked return to the normal shape and texture. This was found true not only for the species of *Marasmius* studied, but also for the three species of *Collybia*. Spore deposits

[2] **Collybia Cookei** (Bres.) comb. nov. is the name proposed for the agaric hitherto recognized as *Collybia cirrata* Fries var. *Cookei* Bres.

were not obtained from any of the revived plants of *Marasmius*. On the other hand, revived plants of two of the species of *Collybia* deposited abundant viable spores. Whether the spores of *C. Cookei* and *C. tuberosa* were produced after revival, or whether it was merely a mechanical shedding of spores already formed, was not determined. This evidence would tend to put this group of *Collybia* species into the section Marasmioidae instead of the section Vestipedes where they have been placed for so many years.

SUMMARY

1. During the course of this investigation, the vegetative growth and sexual reactions have been studied for the following fungi: *Marasmius elongatipes* Peck, *Collybia cirrata* Fries, *C. cirrata* Fries var. *Cookei* Bres. and *C. tuberosa* Fries.

2. These were all found to be tetrapolar species.

3. Strains of *Marasmius elongatipes* from different localities in the vicinity of Ann Arbor, Michigan, were completely interfertile with each other when paired.

4. The kind of diploidization presented by *Marasmius elongatipes* was found to agree with the diploidization phenomenon as described by Buller for *Coprinus lagopus*.

5. A different type of sexual reaction was found in the *Collybia* species studied. Here diploidization is localized, being confined to the region of contact of the paired mycelia. Diploid cells form in this region, and from them the diploid mycelium grows, but the original haploid mycelia are not converted into diploid mycelia. For this, the term "limited diploidization" is suggested.

6. Oidia were found to occur on haploid mycelia of the three *Collybia* species studied but not on the haploid mycelia of *Marasmius elongatipes*. No oidia were seen on diploid mycelia of any of the species studied.

7. All attempts to produce hybrids between the three closely related forms of *Collybia* yielded only negative results.

8. The mycelial fusion test for specific identity was used with these species, and no fusions were observed between the three forms.

9. It is therefore concluded that *Collybia cirrata* Fries var. *Cookei* Bres. should be given specific rank, as *Collybia Cookei* (Bres.) J. Arnold.

10. Sporophore production was obtained upon artificial media for *Marasmius elongatipes*, *M. capillaris*, *M. epiphyllus*, *Collybia cirrata*, *C. Cookei* and *C. tuberosa*.

11. Pilei of *Marasmius* species were revived, but no spore deposits were obtained from them.

12. Pilei of *Collybia* species were successfully revived after more than two months in a dried condition. Basidiospores were discharged from them and germinated in the usual time on nutrient agar.

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LITERATURE CITED

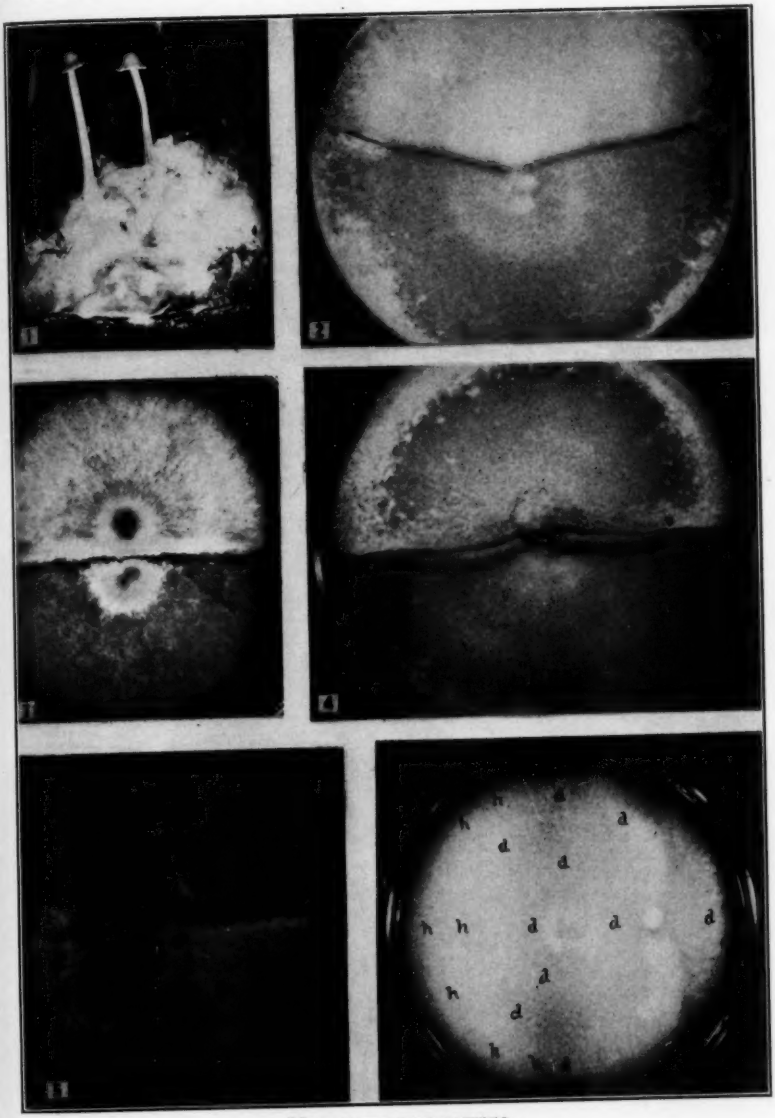
1. **Arnold, Jean D.** A comparative taxonomic study of forms of *Collybia tuberosa* Fr. and *Collybia cirrata* Fr. Papers Mich. Acad. **19**: 55–58. *pl. 1.* 1933. (Published 1934.)
2. **Bensaude, Mathilde.** Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes. (These, Paris), 156. 1918.
3. **Berkeley, M. J.** *Agaricus tuberosus* Bull. (Mycology.—No. XVIII.) Gard. Chron. **1860**: 456. 1860.
4. **Brodie, H. J.** The occurrence and function of the oidia of the Hymenomycetes. (Thesis, in Mss.)
5. **Brunswik, Hermann.** Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*. Bot. Abhandl. 1^5: 1–152. 1924.
6. **Buller, A. H. R.** Researches on fungi **4**: 329 pp. 1931.
7. **Davidson, A. M., Eleanor S. Dowding & A. H. R. Buller.** Hyphal fusions in Dermatophytes. Canadian Jour. Res. **6**: 1–20. 1932.
8. **Etter, B. E.** New media for developing sporophores of wood rot fungi. Mycologia **21**: 197–204. 1929.
9. **Gäumann, E. & C. W. Dodge.** Comparative morphology of fungi. 1–701. 1928.
10. **Hanna, W. F.** The problem of sex in *Coprinus lagopus*. Ann. Bot. **39**: 431–457. 1925.

11. **Kauffman, C. H.** A contribution to the physiology of the Saprolegniaceae. Ann. Bot. **22**: 361–387. 1908.
12. **Kniep, Hans.** Über morphologische und physiologische Geschlechtsdifferenzierung. (Untersuchungen an Basidiomyzeten.) Verh. Physik.-med. Ges. Würzburg. N.F. **46**: 1–18. 1921.
13. ——. Über Geschlechtsbestimmung und Reduktionsteilung. Verh. Physik.-med. Ges. Würzburg. N.F. **47**: 1–28. 1922.
14. ——. Die Sexualität der niederen Pflanzen. 1–544. 1928.
15. **Kotila, J. E.** A study of the biology of a new spore-forming *Rhizoctonia*, *Corticium praticola*. Phytopathology **19**: 1059–1099. 1929.
16. **Lohman, M. L.** A study of *Glonium parvulum* in culture. Papers Mich. Acad. **13**: 141–157. 1931.
17. **Mounce, Irene.** The biology of *Fomes pinicola* (Sw.) Cooke. Canada Dep. Agr. Bull. N.S. **111**: 1–54. 1929.
18. **Oort, A. J. P.** Die Sexualität von *Coprinus fimetarius*. (Thesis Utrecht.) 1930.
19. **Pennington, L. H.** *Marasmius* species occurring in temperate North America. N. Am. Fl. **9**: 252–254; 269–286. 1915.
20. **Poole, R. F.** The fruiting of *Collybia dryophila* in pure culture. Mycologia **20**: 31–35. 1928.
21. **Smith, A. H.** Investigations of two-spored forms in the genus *Mycena*. Mycologia **26**: 305–331. 1934.
22. —— **& H. J. Brodie.** Cultural characters and pairing reactions of monosporous mycelia and development of the fruit body of *Pholiota* (*Flammula*) *polychroa*. Bot. Gaz. **96**: 533–546. 1935.
23. **Vandendries, René.** La conduite sexuelle des Hyménomycètes interprétées par les théories de Hartmann concernant la bisexualité et la relativité sexuelle. Bull. Acad. Belg. 1930.
24. ——. Les polarités sexuelles de *Coprinus tergiversans*. Fr. Bull. Soc. Myc. Fr. **47**: 36–43. 1931.
25. ——. La tétrapolarité sexuelle de *Pleurotus columbinus*. La Cellule **41**: fasc. 3, 267–278. 1932.
26. ——. Nouvelles investigations dans le domaine sexuel des Hyménomycètes. Bull. Soc. Myc. Fr. **49**: 130–165. 1933.
27. ——. De la valeur du barrage sexuel, comme critérium dans l'analyse d'une sporée tétrapolaire de basidiomycète: *Pleurotus ostreatus*. Genetica **15**: 202–212. 1933.
28. ——. Nouveau critère spécifique des Basidiomycètes. Bull. Soc. Linn. Lyon, no. 7, 1–4, Septembre, 1933.
29. —— **& H. J. Brodie.** Nouvelles investigations dans le domaine de la sexualité des Basidiomycètes, et étude expérimentale des barrages sexuels. La Cellule **42**: 165–210. 1933.

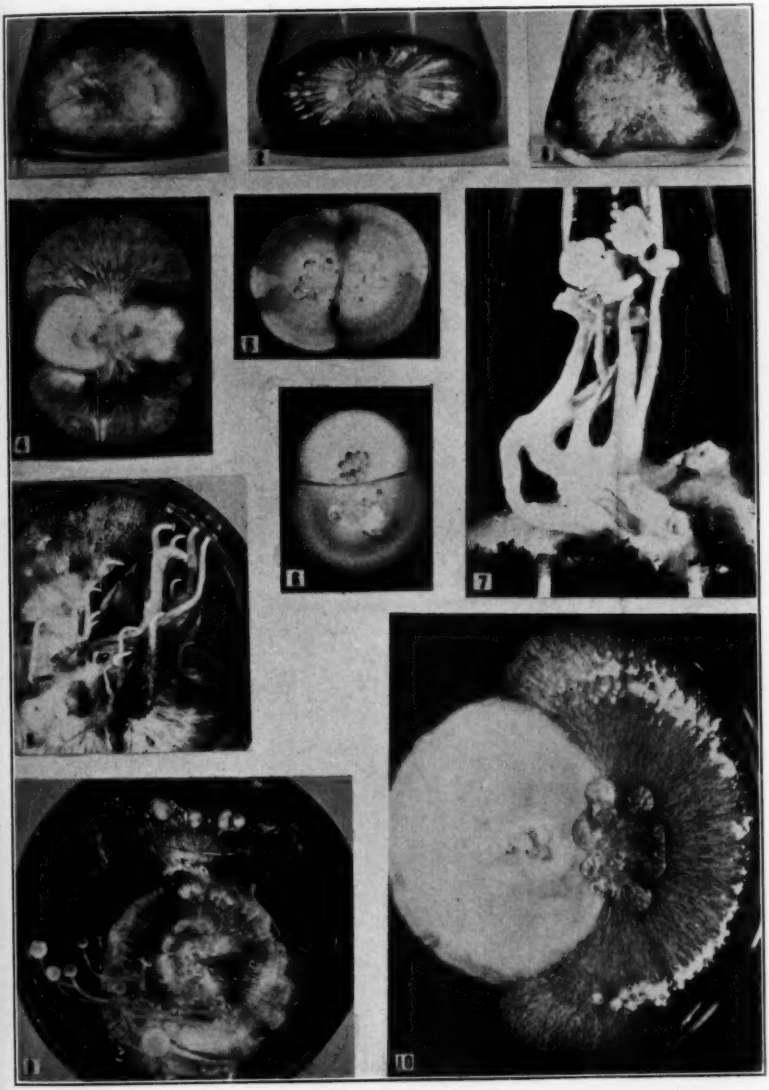
EXPLANATION OF PLATES

PLATE 35

Marasmius elongatipes Peck: 1, sporophore development on medium 5; 2, two incompatible haploid mycelia, showing the line of demarcation between them (3 ab × 16 aB); 3, two incompatible haploid mycelia, showing line of



MARASMIUS ELONGATIPES



COLLYBIA SP.

demarcation between them (2 ab × 20 Ab); 4, two mycelia of the same sex but from different basidiospores, showing line separating the two mycelia (8 AB × 15 AB); 5, two incompatible haploid mycelia, showing line of demarcation between them (14 AB × 26 aB); 6, diploidization as it occurs when a large haploid mycelium (7 AB) is inoculated at the margin with a complementary haploid mycelium (2 ab). Areas already diploid marked d, areas still haploid marked h.

PLATE 36

Fig. 1, *Collybia tuberosa* Fries, showing the development of horn-like sclerotia on *Boletus* extract agar; 2, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.) showing the development of large irregular sclerotia on Kauffman's agar in which *Boletus* extract was used instead of water in the formula; 3, *Collybia cirrata* Fries, development on Kauffman's agar in which *Boletus* extract was used instead of water; 4–6, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.); 4, two haploid mycelia of complementary sexual constitution paired to show how the diploid mycelium forms "Limited diploidization" (15 aB × 17 Ab); 5, two mycelia from the same spore paired to show complete intermingling (8 aB × 8 aB); 6, two mycelia of the same sexual constitution but from two different spores paired to show the line of demarcation between them (15 aB × 16 aB); 7 and 8, *Collybia tuberosa* Fries; 7, development of abnormally large sporophores on *Boletus* pilei laid on top of moist soil; 8, showing sclerotia elongating into stipe, and pilei beginning to differentiate, also showing how sclerotia turn towards light; 9 and 10, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.); 9, development of sporophores on pilei of *Boletus luteus;* 10, diploidization as it occurs when a large haploid mycelium (2 AB) is inoculated at the margin with a complementary haploid (3 ab), diploid mycelium gradually growing around haploid. (Contrast with figure 6 on previous plate.)

THE MECHANICS OF SEXUAL REPRODUCTION IN NEUROSPORA

B. O. DODGE

(WITH PLATES 37–40)

The course by which a perithecium of *Neurospora* reaches full maturity is marked by three rather distinct stages of development. First there appears the perithecial fundament, the ascogonium. This is quickly surrounded by several layers of compact hyphal growth to form the incipient ascocarp. Nuclei of opposite sex reaction then come together in the ascogenous cells if these cells do not already contain both kinds, as is normally the case in *N. tetrasperma*. This stimulates further growth of the fruit body, the differentiation of wall tissue, the formation of the ostiolar papilla, and finally the development of ascogenous hyphae. Fertilization is consummated with nuclear fusion in the ascus. This is followed by the reduction divisions and the delimitation of ascospores.

THE INCIPIENT PERITHECIUM

The ascogonium is of the type usually found in species of the Sordariaceae figured many times in the early literature on the subject. It consists of a rather blunt-ended coiled structure of five or six cells or more. Each cell contains several nuclei. The ascogonium of normal *N. tetrasperma* where the cells are provided with nuclei of both sexes from the start, never differentiates a trichogyne (**4**). Basal cells and branches from the parent hypha grow out to encompass the ascogonium, surrounding it with a compact tissue of several cell layers (PLATE 37, *I*).

The ascogonia of the heterothallic species and of the unisexual races of *N. tetrasperma* are all very much like those formed on mycelia of normal bisexual *N. tetrasperma*. They consist of the same blunt-ended coils of two or three irregular turns composed of from five to ten cells and without any trichogynes whatever. Just what the large Y-shaped branches one sees in many sections

(PLATE 38, *E*) represent is a question. The cells contain several nuclei often arranged in pairs. The pairs represent merely sister nuclei. Sterile haploid hyphal branches interlace to form a compact weft about the coil. After the incipient perithecium has reached a certain size just visible to the unaided eye, it usually ceases to increase further if the race is grown by itself. Soon, however, trichogynous elements grow out from certain of the ascogonial cells. These narrow, deeply staining branches burrow out through the surrounding compact sterile tissue, zigzagging as they pass between the cells as though they were the hyphae of some intercellular parasite. They are somewhat like the trichogynes of *Coccomyces* so well described and figured by Backus (**3**). They are sometimes forced to grow around some distance in a circle before finding a place to emerge (PLATE 37, *E*). More frequently these hyphae grow out as branches from the end cells of the ascogonium, but they may develop from a more central cell. The tip end may form two or three such receptive branches.

The basal part of the ascogonium is apt very early to send out one or two branches that maintain direct connection with the outside. Such branches take the stain deeply and no doubt can act as receptive elements. They are sometimes partly inclosed by the incipient perithecial tissue (PLATE 38, *G*). At other times they are free from the point of their origin outward.

Photographs of sections of several incipient perithecia of *Neurospora sitophila* showing the least complicated types of ascogonia are reproduced in plates 37 and 38. These were developed on unisexual mycelia grown separately. The details are given in the plate legends. There can be no doubt that when one applies either the orange-colored monilioid conidia or the microconidia artificially, these trichogynous outgrowths are the means by which nuclei of the other sex are brought into the ascogenous cells. Looking down on plate cultures one often sees these long trichogynes extending out into the air or bending down, their tips fusing with the ordinary vegetative hyphae. No doubt in mixed cultures this would result in fertilization.

At the Sixth International Genetics Congress held in Ithaca, N. Y., 1932, the writer demonstrated with enlarged photographs the course of development of incipient perithecia at six-hour

intervals, beginning with the hour at which the monilioid conidia had been applied to the surface of the young fruit bodies. Two of this series of photographs were published in Torreya (11) where it was pointed out that the appearance of the hair-like outgrowths is such as to suggest that they may be receptive hyphae. The photographs exhibited at Ithaca (together with this statement) seem to have misled certain observers into thinking that all such outgrowths must be receptive hyphae. Sections show conclusively that in *Neurospora* most of these delicate hairs grow out from the outermost sterile wall cells and have no direct connection whatever with the ascogonium (PLATE 37, *I*). It is probably the same in *Pleurage anserina*.

The details concerning the formation of spermatia or microconidia by species of *Neurospora*, their germination to form perfectly normal mycelia and also their function as fertilizing elements will be found in earlier papers (**6–8**). It has also been shown that the monilioid conidia serve much more efficiently as spermatizers than do the microspores themselves, and recent experiments indicate that young aerial hyphae, and even trichogynes or trichogynous hyphae if they come in contact with trichogynous elements of the opposite sex reaction, are capable of effecting fertilization It is not very difficult to make these incipient perithecia fertilize each other.

UNISEXUAL MYCELIA UNITED VEGETATIVELY

It remains to determine whether, in mixed cultures of heterothallic races, perithecia mature only when nuclei from outside fertilizing elements such as microspores and conidia enter the ascogonia by way of trichogynes; or may it be that, following vegetative hyphal fusions, there then arise perithecia whose ascogonia are provided with nuclei of both sexes because of nuclear migrations, so that trichogynes are not needed and are not formed.

Köhler (**14**), who worked rather extensively on anastomoses, found no evidence that in *Neurospora* one could obtain a bisexual mycelium by mixing unisexual conidia of both sexes. The germ tubes would fuse and the regeneration hypha would show a plasma-streaming, but he could not prove a migration of nuclei.

Lindegren (**15**) without referring to Köhler's work claims on the contrary that such a heterokaryotic condition in *N. crassa* must sometimes result from sowing many ascospores together, but he adds that the bisexual condition may split up again to develop separate unisexual growths. Although one experiment with nonconidial races of *N. sitophila* will be described later on, further work is still necessary before the question as related to normally heterothallic species is settled. The present study is concerned with the same question but more as related to bisexual (facultatively heterothallic) species such as *N. tetrasperma*, *Pleurage anserina* and *Gelasinospora tetrasperma*. Dowding (**12**, **13**) has shown by her excellent work with the last two species that they are like *Neurospora tetrasperma* in that one can readily obtain unisexual races by germinating the small ascospores. No one has hitherto proved that such unisexual races can, by uniting vegetatively, give rise to the normal bisexual type of mycelium containing both kinds of nuclei.

When our tester races S6 and S1, or S9 and S1, which are unisexual components of *N. tetrasperma*, are planted on opposite sides of a plate culture, the fruit bodies are usually first formed where the mycelia meet, after which they appear one by one along the lines of radiately growing hyphae of the strain S6 (**6**). The distribution pattern of course varies greatly, depending on culture conditions. In general, however, the perithecia are formed on the S6 side (**6**, *fig. 3*, and **7**, 1931, *pl. 17*, *fig. 15*, *17*) and not more on the S1 side as stated by Colson (**4**). Dowding (**13**, *pl. 11*, *fig. 11*) shows exactly the same distribution of ascocarps in plate cultures of *Gelasinospora*. This suggests at first a differentiation of the sexes, but it probably means merely the manifestation of genetic factors, some of which may possibly be sex-linked. This point will be discussed later on. In any event, why is it that fruit bodies of these species tend to be distributed in this curious fashion, over one side of the plate, often confined to a V-shaped sector at first? There are hundreds of anastomoses along the line of meeting and it looks as though the nuclei coming over from the S1 mycelium may migrate down through the S9 hyphae. In that case the ascogonia of the new incipient perithecia forming on these hyphae, now bisexual because of the

migration, would contain both kinds of nuclei so that the perithecia would go right on and mature. In order to obtain some definite information on this point a number of culture experiments were carried out. A brief outline of the principles involved will facilitate a better understanding of the results to be reported.

The fact that in plating out microconidia and monilioid conidia from the S9 side of plate cultures after perithecia are formed there, one finds all of his isolates belonging to sex B and not any to sex A, or that all are sex A and none sex B, or again that both sexes are represented separately, does not prove anything particularly as regards nuclear migration or intermingling of mycelia. With only nuclear migration operating to bring the other kind of nuclei also into some cells of the S9 mycelium we could still have the split-up again. It is only when we obtain *bisexual* conidia or *bisexual* hyphal tips, do we know that the anastomoses have resulted in bringing together both kinds of nuclei in hyphal cells. In any mixed culture, as previously noted (**5, 10**), if new branches arise from vegetative fusions to form bisexual conidia, such conidia must be greatly outnumbered by the unisexual conidia already in the culture so that it would be difficult to find them.

Nuclear migration in plate culture

Tester races S3 and S9 were grown from opposite sides of a plate. Perithecia began to form as usual about the fourth day on the S9 side of the line of meeting. Four small blocks of agar bearing a few very young perithecia were then transferred to separate plates which were incubated for twelve hours. The hyphae grew out so that single hyphal tips could then be isolated very easily. Seven 1-tip tube cultures were obtained from each of the four transplants. Later they all produced perithecia, proving that the single tips isolated must have carried both kinds of nuclei. Since mycelia S3 and S9 with which the plate was inoculated are both unisexual, the bisexual tips isolated later from the S9 side must have represented new branches that grew out from the old S9 mycelium after it had received the S3 nuclei passed along down following anastomoses at the line of meeting. It may be claimed, however, that the bisexual fusion cells result-

ing from anastomoses could proliferate to form a *new* mycelium which would spread out over or through the old unisexual mycelia already in the plate. In such a case there is no reason why this new bisexual mycelium should grow out only over the S9 side. In any case one should obtain at least as many unisexual 1-tip isolates like S9 as he does bisexual tips. It is a very remarkable fact that not one of the twenty-eight 1-tip isolates was unisexual. Furthermore if new bisexual growth does proceed from points of anastomoses one should be able to isolate bisexual conidia very readily from such plates, which is not the case. It is more reasonable to assume that nuclei from the S3 side, following anastomoses with S9 branches, migrate down the S9 hyphae. Ascogonia containing both kinds of nuclei would arise and perithecia would quickly begin to mature.

U-tube cultures

Conclusive evidence of nuclear migration was also obtained from U-tube cultures being carried on for another purpose. The U-tubes employed in this work were the same that were used by the writer (**7**), and by Aronescu (**1**, **2**). The procedure is as follows. Two unisexual races such as S1 and S9 are grown from opposite sides of a plate culture. The two mycelia meet and their hyphal tips anastomose. In two or three days one sees the beginnings of perithecia that are destined to mature ascospores because in some way both kinds of nuclei have been brought together in the ascogonial cells. These young perithecia present a different appearance from that of the unfertilized incipient perithecia always formed on unisexual mycelia. A small bit of agar bearing a few young perithecia is transferred to one arm of the U-tube. Within three or four days many more fruit bodies begin to develop in this arm. In the meantime the mycelium will usually have grown down around into the other arm of the U-tube.

The reader is referred to a preceding paper (**10**) for the pedigree of races used in the following experiments. Unisexual races 38 (9.7C8 × S9) and 26 (9.7C8 × S9) both carry the lethal, l, so that perithecia arising from their mating produce asci but no ascospores. Such races are desirable in this work because no

matter how old a culture may be no ascospores can be formed to germinate *in situ* to mislead one. That is, if one grows these two unisexual races in a mixed culture and at any time succeeds in isolating a *bisexual* conidium or a *bisexual* hyphal tip from that mixed culture he can be sure that nuclei originally carried in separate mycelia had come into the same cells following vegetative fusions or anastomoses. This is the particular point proved by the following culture experiments.

Races 38 and 26 (the parental formula 9.7C8 × S9 may be omitted) were grown from opposite sides of a plate culture. Four days later the mycelia had met and perithecia were appearing on the 38 side of the line. A small block of agar carrying a few of the little fruit bodies was transferred to one arm of a U-tube. In due time more perithecia appeared in this arm and the mycelium passed around into the second arm where more conidia and perithecia with asci eventually matured. In no case were any ascospores delimited, because as stated above both parents carry the lethal. About two weeks from the time the culture was started conidia from the second arm were sowed and allowed to germinate slightly. Twelve 1-conidium tube culture isolates were made, choosing the conidia that germinated first. Twenty more 1-conidium isolates were planted in plates to check against the possible accidental transfer of microconidia. The next day twenty-five 1-tip isolates were made from these plate transplants. As a double check the first transplants were again transferred to a harder agar where no water of condensation could possibly carry microspores over on the hyphal tips to be isolated. By the following day the new growth had formed a circle about 2 cm. in diameter in each case. From each of ten such colonies four 1-tip isolates were made with great care. The results obtained in this experiment show that of the twelve 1-conidium cultures first isolated, eight were probably unisexual and sterile and the other four were certainly bisexual and fertile, showing good perithecia. Of the first set of thirty-eight 1-tip cultures, thirty were unisexual and the other eight showed perithecia. The forty-three doubly checked 1-conidium cultures showed about the same percentage of fertility, thirty-five remaining sterile and so must have been unisexual, and eight formed

perithecia and must have been bisexual. Of the ninety-three cultures making up the three sets, seventy-three remained sterile, and twenty were fertile; therefore their mycelia must have been bisexual. This proves conclusively again that a bisexual mycelium can be obtained as the result of a vegetative union or anastomosing of hyphae from two unisexual mycelia of opposite sex reaction.

Twenty-two of the sterile isolates mentioned above were tested for their sex reaction. All of them were sex B, the same as the no. 26 parent which carries the factor O for orange-colored masses of conidia. The no. 38 parent produces only a few conidia comparatively, so that out of the small number one might not obtain any that would be of reaction type A. As a further check, conidia produced in one of the 1-conidium fertile cultures described above were then sowed, and fifty-one 1-conidium isolates were obtained by Dr. S. M. Pady, to whom the writer is indebted for assistance in checking this work. Twenty-five proved to be bisexual, and twenty-six unisexual and sex B in their reaction. No doubt one could build up a still higher percentage of bisexuality by selection.

The same type of U-tube experiment was then performed but using the unisexual parents 43 (9.7C8 × S9) and T100 (9.7C8 × S9), races carrying the factors aOL and AoL respectively. Normal asci with spores are formed in such matings, so that these experiments should be carried on before ascospores are discharged. A plate was inoculated on opposite sides with the two races 43 and T100. The mycelia soon met and produced perithecia. In order to avoid carrying over ascospores that might germinate, fragments of mycelium were taken from between points bearing perithecia. The fragments were thoroughly washed, and then placed on the agar in one arm of a U-tube. New perithecia soon appeared, and the mycelium grew around into the other arm where more perithecia and conidia developed. Conidia from the top of the agar slant in the second arm were sowed. Fifteen single conidia were isolated and grown in plate cultures as before. The next day one hundred 1-tip cultures representing the fifteen original conidia were made. At the end of ten days only one of these cultures showed perithecia. Twenty

of the sterile cultures were tested for their sex reaction. They all proved to be sex B like the parent no. 43. The results would suggest that unisexual hyphae like the parent 43 had split off from the bisexual mycelium soon after the latter had grown around into the second arm. Race 43 produces many more conidia than does T100 so that only one kind happened to be isolated. That nuclei of both sexes actually reached the second arm is proved by the fact that perithecia were formed there. The two kinds of mycelia might have grown around separately from the beginning, however, but this is not likely.

The experiment was repeated using the same parent races. This time of the twenty-three single conidia isolated thirteen were proved to be bisexual. A third test of the same kind was made again using races no. 43 and T100 as parents. Sixty-one 1-conidium isolates were obtained. Thirty-four of these were proved to be bisexual and twenty-seven unisexual. When grown on dextrose agar twenty-six of the unisexual isolates showed the bright orange-colored masses of conidia characteristic of parent no. 43. Only one isolate, no. C3, looked like the T100 parent which produces comparatively few conidia. This one was proved by test to be sex A, while the other twenty-six were sex B. These results prove again that bisexual conidia can be obtained by combining two unisexual races vegetatively.

Using the tester races S9 and S1 as parents U-tube experiments of the same sort were carried out. The two races were planted on opposite sides of a plate culture December 13. The mycelia had met across the center at the close of the third day. A bit of agar carrying anastomosing hyphal branches at the line of meeting was then transferred to a U-tube. The mycelium had grown around into the second arm by Dec. 19 and conidia as well as young perithecia were being developed. Thirty-five 1-conidium isolates were made from the conidia in the second arm. Twenty-nine of these were grown in tubes and six in plates. By Dec. 25, twenty-five of the tube cultures showed perithecia, the other four were still sterile. As soon as a few hyphal tips had grown out from the six plate transfers, twenty-three 1-tip isolates were made from the transplants which represented six different conidia. The results in this case showed that three of the original conidia were bisexual and three were unisexual.

In case of another experiment with the same parents, S1 and S9, twenty-eight 1-conidium isolates were obtained from conidia taken from the second arm. Only fourteen of these were bisexual. Since thirty-nine of the sixty-three single conidia originally isolated from the second arm of the U-tubes were proved to be bisexual, it is clear that by growing our unisexual tester races S9 and S1 together one is able to unite them vegetatively to give rise to a bisexual mycelium. The bisexual condition due to nuclear migration must have been fairly complete at the points from which the transplants were taken from the original plate culture.

In the course of this work over one hundred U-tube cultures of *N. tetrasperma* were studied. In every case but two, when perithecia matured in the first arm the mycelium that grew around into the second arm matured perithecia there also. There appeared to be a correlation between the number of fruit bodies developed in the two arms as though in some cases the heterokaryotic condition was more complete in some transplants than in others. Whether this represents a difference in rates of nuclear division or not is still a question.

Aronescu (1) incidental to her work on possible hormone action in sexual reproduction in case of *Neurospora sitophila* made eighty-six U-tube cultures in which she inoculated one arm of each tube in two places with conidia or bits of mycelium of opposite sex. In each case she found that while perithecia matured in the arm inoculated, only one kind of mycelium grew around into the other arm. No perithecia ever developed in the second arm. This would mean that unisexual mycelia of normally heterothallic races do not unite so readily to bring about a heterokaryotic condition and that the first mycelium to get started down the U-tube would be the only one to reach the other arm because it would use all of the oxygen available. Further work along the same line with both *N. sitophila* and *N. tetrasperma* will be necessary before the differences in behavior are understood. To make the tests comparable, parallel experiments must be made using, in one set, two kinds of conidia inoculated separately, and in the other allowing opportunity for hyphae of opposite sex to intermingle and use some of the mixture as inoculum.

Neurospora sitophila

One of the most interesting questions regarding the *Neurospora* species, as Lindegren (**15**) has pointed out, is the difference between those species like *N. sitophila* and *N. crassa* which are normally heterothallic and *N. tetrasperma* and *N. Toroi* which are normally bisexual and only facultatively heterothallic. Normal bisexual mycelia of the last two species form some unisexual conidia in culture and we have proved beyond question that unisexual mycelia derived from such conidia can be reunited readily by vegetative fusions to give the normal bisexual type of growth. The mechanism by which bisexual ascospores are cut out in these species and unisexual ascospores in case of *N. sitophila* is very effective. Is it merely the working out of the mechanism which determines the distribution of nuclei, or may there not be something more fundamental back of it, something which holds nuclei of opposite sex together in the same cells in *N. tetrasperma*, but keeps them apart in *N. sitophila* until a state of maturity is reached that demands their association in the phases of sexual reproduction? No one has heretofore described an experiment adequately proving that in *N. sitophila* or *N. crassa* bisexual mycelia can be obtained by vegetative fusions of unisexual hyphal branches or by fusions between germinating conidia or ascospores. As noted previously Köhler was not able to obtain such bisexual mycelia in this way. The following experiment would seem to confirm Köhler's report.

Races 56.2 and 56.6 of *N. sitophila* are non-conidial unisexual races. They were grown in plate cultures from opposite sides. Most of the perithecia were formed on the 56.6, sex A, side of the cultures. From each of eight different plates a small block of agar bearing a few immature but fertilized perithecia were transferred to separate plates. After new growth had developed about two centimeters in diameter, seventeen 1-tip isolates were made from each of the eight plates making in all 136 isolates. In similar experiments with *N. tetrasperma* and *Gelasinospora tetrasperma* reported in this paper every one of such isolate cultures produced perithecia. In exactly the same type of experiment with *N. sitophila* not a single 1-tip isolate produced perithecia with asci. This proves conclusively that in such a mating

heterokaryosis is certainly not readily brought about by vegetative fusions and nuclear migration. Moreover one can trace some hyphae from the 56.2 side well over on the 56.6 side of the plate, and some perithecia are usually formed on the 56.2 side also.

Gelasinospora tetrasperma

The writer is indebted to Dr. Dowding (Mrs. E. Silver Keeping) for cultures of her beautiful species *Gelasinospora tetrasperma*. The best possible proof that one can obtain a bisexual mycelium by the vegetative union of two unisexual races was obtained with this species. Our unisexual races Gel. 1 and Gel. 5 were grown from the opposite sides of a plate culture for two or three days. At the end of this time the mycelia had met at the center where hyphal fusions occurred abundantly. As soon as one could see that some perithecia were forming on the Gel. 5 side six small blocks of agar were cut out from various regions on that side of the line. These were transferred to individual plates. The hyphae were allowed to grow out so that single hyphal tips could be isolated readily. Every one of the thirty-eight 1-tip cultures so obtained matured perithecia with ascospores.

The same kind of test for nuclear migration was carried out using races of the unisexual Gel. 7 and Gel. 11 as parents. Here the perithecia always form on the Gel. 11 side of the plate. Four transplants taken from different parts of the area bearing young perithecia were made to separate plates. After new growth had become well established seven 1-tip isolates were obtained from new growth from each of the four original transplants. This time twenty-five of the 1-tip isolates produced perithecia abundantly, but three isolates from one transplant matured only a few fruit bodies at first, showing that heterokaryosis was not yet complete.

Later experiments proved that one need not wait until perithecia are formed before making the transplants. Nuclear migration following hyphal anastomoses here seems to take place rather rapidly. There is no evidence whatever in our cultures on 2 per cent Difco corn-meal agar plates that the mycelium from one side runs over on the other side; if it does one should obtain more unisexual 1-tip isolates. Neither microconidia nor

other asexual spores are formed in cultures of *Gelasinospora* according to Dowding, and such bodies were not seen in any of our cultures. When a unisexual clone is grown alone one can see what look to be long trichogynes emerging from ascogonia within the incipient perithecia. If a weft of young aerial hyphae from a clone of opposite sex reaction type should come in contact with these trichogynes the incipient perithecia would no doubt go on and mature.

ASCOCARP DISTRIBUTION PATTERN IN NEUROSPORA AND GELASINOSPORA

Lindegren (**15**) has proved conclusively that the genes for "pale" and "non-pale" and for the sex-reaction factors (+) and (−) are linked. Zickler (**22**) has also a very striking sex-linked character in a species of *Sordaria* which he calls *Bombardia lunata*. He finds that the factors for color of the ascospores in certain crosses are linked with the sex reaction factors. This enables him to study the first- and second-division segregations to great advantage.

More recently the writer (**10**) working on the effects of irradiation of ascospores of *Neurospora tetrasperma* and describing the effects of a recessive factor, l, lethal for ascospore delimitation when homozygous, pointed out that this same factor, l, seems to suppress the action of a factor O for bright orange-colored masses of conidia. It was suggested at that time, and it has since been proved more conclusively by Tai (**21**), that the factor O is linked with the sex-reaction factors.

Neurospora tetrasperma

The peculiar distribution of ascocarps in plate cultures of *N. tetrasperma* when one grows the unisexual component races from opposite sides of a plate culture (**6**, **8**), referred to previously, p. 421, is such as to suggest the possibility of another linkage involving the sex-reaction factors. The writer has at various times isolated a number of unisexual clones by germinating the small ascospores. Mr. F. L. Tai and Dr. S. M. Pady working in our laboratory on other problems have turned over similar clones. When these clones are mated in plate cultures one finds a corre-

lation between the sex-reaction types and the manner of distribution of the perithecia. This is brought out rather easily in large tube cultures with long slants (PLATE 39, *B*). The top of the slant can be inoculated with mycelium or conidia of one sex and the bottom of the slant with the other.

Seven sex A clones were mated with three sex B clones in all possible combinations. The distribution pattern was very strikingly correlated with the reaction type, with one exception, fruit bodies forming on the sex A side. Clone S23 may represent a cross-over if there is a linkage. These cultures were kept several months but the patterns were not changed.

We have paired some twenty-one clones of sex A reaction with eighteen clones of sex B type in plate cultures. In some instances the distribution pattern was not absolutely fixed, but it was sufficiently constant to suggest that the distribution of perithecia in such matings may be genetic and possibly linked with the sex factors. There appear to be three types of distribution patterns. The first, figured in papers referred to previously, where the ascocarps were very definitely located on the sex A side of the meeting line with none of them on the B side. Then we had a few cases with the same but reverse pattern, the fruit bodies being well spread out on the sex B side with few or none on the sex A side. In the third series a few large perithecia were scattered down along the line of growth on the B side. Across the meeting line on the A side there was a region where the perithecia were so crowded as to make a black zone whose margin on the sex B side was very clearly marked at about the line of meeting, but where the margin on the sex A side was rather corrugated well out on the A mycelium as though nuclear migration was going in that direction but very slowly and completely as far as it goes (PLATE 40, *B*). It must be admitted that as between various combinations some interactions are more decided than others (PLATE 40, *A*). It is clear that while the distribution pattern has a genetic basis it is not determined solely by a sex difference or by a single pair of factors.

Neurospora Toroi

Our two unisexual strains, Toro A and Toro B of *Neurospora Toroi* Tai (**21**) grown together, originally produced most of the

fruit bodies on the A side. This species does not produce fertile hybrids readily with *N. tetrasperma*, but it does react very positively with it so that we can readily classify the races as to their sex reactions. The other interspecific reactions with this species in all cases agree, and the Chinese species *N. intermedia* Tai (**21**) does produce fertile hybrids with *N. Toroi*, so that we know that the clones one may isolate from the five species of *Neurospora* now being grown in culture all fall into one or the other of two sexual groups. When our albino non-conidial races 56.2 and 56.6 of *N. sitophila* are mated in plate cultures the most fruit bodies are generally found on the 56.6 side. It is very doubtful, however, that the distribution pattern regularly follows the reaction factors through such a wide range.

Gelasinospora tetrasperma

Reference was made previously (p. 421) to the very striking resemblance between *Neurospora tetrasperma* and *Gelasinospora tetrasperma* in the way the ascocarps are distributed over the cultures where heterothallic clones are mated. The writer isolated nine unisexual 1-ascospore clones from material generously sent him some time ago by Dr. Dowding. When these were mated in plate cultures in all possible combinations it was found that clones Gel. 4, -5, -9 and -11 were of one sex reaction and Gel. 1, -2, -3, -7 and -8 were of the opposite kind. With one exception the fruit bodies were very definitely distributed on the side of the clones in the first group (PLATE 39, *A*). If the distribution pattern is sex-linked, clone Gel. 3 may represent a cross-over spore because in the matings with this clone the fruit bodies were developed more or less on both sides of the line. The final distribution will be determined by the resultant effect of the opposing forces. If the progeny represented in the ascus in which Gel. 3 spore was formed had all been analyzed the reciprocal spore would have been obtained. If the distribution pattern is sex-linked, clone Gel. 3 should be mated against a cross-over to give a definite reverse pattern.

In another set of eight unisexual clones three cultures differed from the other five in their somewhat darker color and their fluffy white aerial hyphae and slower growth. With the idea

that the three were of the same sex and opposite to that of the others, they were grown separately against the other five. The results proved the correctness of the prediction. The line of meeting in each plate was well over on one side, but the fruit bodies in each case were distributed on the side of the more rapidly growing race. This proves that the direction in which the nuclei migrate the most readily is not necessarily correlated with the direction of growth of the most vigorous mycelium as has been observed in some other cases.

In such studies each clone should be tested against a particular tester strain because other genetic factors as well as unfavorable culture conditions must enter to disturb the distribution pattern. On 2% Difco corn-meal agar under the conditions of our cultures the pattern is rather constant and such as to suggest a linkage of some sort.

Colson (**4**, p. 217) says concerning the distribution of fruit bodies in cultures of *Neurospora tetrasperma:* "When inoculations of A and B are made on opposite sides of a petri dish, each strain grows completely across the medium so that the two strains become intimately mixed. As a result of this the perithecia do not form in a line at the meeting of the two mycelia but are scattered over the whole surface." The writer has not found any evidence for such behavior in his cultures of this species on corn-meal agar as we make it. If the nutrient medium contains too much starch or unfiltered corn-meal, or if one uses a 4% instead of our 2% agar medium then one might find the distribution pattern greatly altered because aerial hyphae then tend to grow out over the surface more generally. If water of condensation flows around in the plate, conidiation will affect the pattern. The linkage between the factors for bright orange-colored masses of conidia and sex-reaction factors is not very manifest on 2 per cent Difco corn-meal agar, but it is very striking in cultures on dextrose agar. So it is with the distribution pattern; to bring it out best one must have certain definite culture conditions.

Colson referring to first division versus second division segregation assumes that when a normal ascospore of *N. tetrasperma* taken from a four-spored ascus is proved to be unisexual this denotes a cross-over. Dodge, she says, never reported a cross-

over in his genetic studies. If isolating normal sized spores that are unisexual means finding a cross-over Lindegren and the writer have both reported such an event several times, and Kniep and Lindegren have diagrammed asci showing how this could occur. Second division segregation means a cross-over only when it is proved that in *N. tetrasperma* the spindle-fiber attachment points always reduce in the first division. Zickler, as noted previously, in order to explain his results has to assume otherwise in case of *Bombardia*. Nevertheless Colson's suggestion is a good one because if it is correct it gives us an additional means of analyzing linkages in these facultatively heterothallic species.

Zickler (**22**) claims to have what approaches one case of tetrapolar sexuality in *Bombardia*. His clones **A** lan. and **a** lan. are of opposite sex reaction. Since these clones produce very few incipient perithecia they are not only self-sterile, he says, but they are also cross-sterile. Clones **a** bulb. and **A** bulb. both produce incipient perithecia and also microconidia so that they are reciprocally cross-fertile. He finds that he can successfully "spermatize" clone **a** bulb. with clone **A** lan. but not clone **A** lan. with clone **a** bulb., and so with the other pair. He leaves one with the impression that while clones **A** lan. and **a** lan. can be used as spermatizers they can not be made to produce perithecia because they do not form ascogonia. We have not as yet been able to obtain these races from him for experimentation, so that it is impossible to say definitely what would happen if his races **A** lan. and **a** lan. were grown together in the same culture. There is the possibility, judging from what we find in similar cases in *Neurospora* and *Gelasinospora* (see also Aronescu, 1) that Zickler's races **A** lan. and **a** lan. are not cross-sterile as he leaves one to infer. Following hyphal anastomoses in a mixed culture incipient perithecia might very well arise and go on to maturity. In case of *Gelasinospora* where there are no microconidia spermatization is impossible, and we have furthermore proved that bisexual races readily result from anastomoses followed by nuclear migrations.

Lindegren (**15**) assumes that in nature where ascospores of *N. crassa* are discharged close together the mycelia arising from their germination immediately anastomose so that nuclei representing

different genotypes commingle in a coencytium which later becomes septate. Such a resultant mycelium being bisexual would, he says, produce perithecia were it not for sterility factors so abundant in these natural strains. He does not report having proved this by experiment and our experiment with *N. sitophila* reported above does not support this argument. He accepts the Moreau-Moruzi (**17**) hormone hypothesis without testing it out or without referring to work (**27**) disproving such an action. Zickler (**22**) has also proved the fallacy of this hormone idea. Stevens (**19**) claims to have induced self-sterile ascomycete mycelia to produce perithecia on exposure to ultra-violet light. Lindegren thinks that Stevens was working with strains which were bisexual heterokaryons. On the other hand, the writer does not question the correctness of Lindegren's view that in *Neurospora crassa* one might find in nature races that are heterokaryotic (temporarily?) containing nuclei of opposite sex, yet tending to remain self-sterile because of incompatibility factors. Such a condition in *N. tetrasperma* hybrid offspring had already been reported (**8**, p. 20–26), where races tended to remain sterile when grown alone, but when mated separately with the tester strains of opposite sex produced perithecia in both matings. If one did not know the nuclear story he would insist that the tester strains were not unisexual but were merely self-incompatible and cross-compatible in their reactions. Similar incompatibilities often exist in *Gelasinospora*. Our unisexual race Gel. 13 is very fertile with races Gel. 15 and Gel. 17, but is very incompatible with Gel. 18 and Gel. 19; in fact, so far it is rather cross-sterile with these two although proved beyond question to be of opposite sex reaction.

Further experiments with *N. sitophila* to be reported in another connection show that this normally heterothallic species is fundamentally very different from those facultatively heterothallic species like *N. tetrasperma* and *Gelasinospora tetrasperma*.

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LITERATURE CITED

1. **Aronescu, A.** Further studies in *Neurospora sitophila*. Mycologia **25**: 43–54, *pl. 14*. 1933.

2. ——. Further tests for hormone action in *Neurospora*. Mycologia **26**: 244–252, *pl. 30*. 1934.
3. **Backus, M. P.** Initiation of the ascocarp and associated phenomena in *Coccomyces hiemalis*. Contr. Boyce Thompson Inst. **6**: 339–379, *f. 1–8*. 1934.
4. **Colson, B.** The cytology and morphology of *Neurospora tetrasperma* [Shear &] Dodge. Ann. Bot. **48**: 211–224, *pl. 4, 5*. 1934.
5. **Dodge, B. O.** Unisexual conidia from bisexual mycelia. Mycologia **20**: 226–234, *f. 1*. 1928.
6. ——. Material for demonstrating sexuality in the ascomycetes. Torreya **30**: 36–40, *pl. 2, f. 1–3*. 1930.
7. ——. Heterothallism and hypothetical hormones in *Neurospora*. Bull. Torrey Club **58**: 517–522, *f. 1*. 1931.
8. ——. Inheritance of the albinistic non-conidial characters in interspecific hybrids in *Neurospora*. Mycologia **23**: 1–50, *pl. 1–7*. 1931.
9. ——. A lethal for ascus abortion in *Neurospora*. Mycologia **26**: 360–376, *pl. 42–44, f. 1, 2*. 1934.
10. ——. A recessive factor lethal for ascospore formation in *Neurospora*. Bull. Torrey Club **62**: 117–128, *pl. 9, 10*. 1935.
11. —— **& Swift, M. E.** A simple way to demonstrate sexual reproduction in the bakery mold, *Neurospora*. Torreya **33**: 31–37, *f. 1–3*. 1933.
12. **Dowding, E. S.** The sexuality of the normal, giant, and dwarf spores of *Pleurage anserina* (Ces.), Kuntze. Ann. Bot. **45**: 1–14, *pl. 1, f. 1–10*. 1931.
13. ——. *Gelasinospora*, a new genus of Pyrenomycetes with pitted spores. Canadian Jour. Res. **9**: 294–305, *pl. 1–3, f. 1–8*. 1933.
14. **Köhler, E.** Zur Kenntnis der vegetativen Anastomosen der Pilze. II Mitteilung. Ein Beitrag zur Frages der specifischen Pilzwirkungen. Planta **10**: 495–522, *f. 1–30*. 1930.
15. **Lindegren, C. C.** The genetics of *Neurospora*—III. Pure bred stocks and crossing-over in *N. crassa*. Bull. Torrey Club **60**: 133–154, *pl. 9, f. 1–6*. 1933.
16. ——. The genetics of *Neurospora*. V. Self-sterile bisexual heterokaryons. Jour. Genetics **28**: 425–435. 1934.
17. **Moreau, F. & Moruzi, C.** Recherches experimentales sur la formation des périthèces chez les "*Neurospora*." Comp. Rend. Acad. Sci. Paris **192**: 1475–1478. 1931.
18. **Moruzi, C.** Sur une maladie du Champignon de couche causée par un *Monilia*. Bull. Soc. Myc. Fr. **46**: 143–148, *f. 1–4*. 1930.
19. **Stevens, F. L.** Effects of ultra-violet radiation on various fungi. Bot. Gaz. **86**: 210–225. 1928.
20. **Tai, F. L.** Sex reaction linkage in *Neurospora*. In press. 1935.
21. ——. Two new species of *Neurospora*. Mycologia **27**: 328–330. 1935.
22. **Zickler, H.** Genetische Untersuchungen an einem hetero-thallischen Askomyzeten (*Bombardia lunata* nov. spec.) Planta (Arch. wiss. Bot.) **22**: 573–613, 1934.

EXPLANATION OF PLATES

Photographs mounted to show original orientation unless stated otherwise.

PLATE 37

Neurospora sitophila race 56.2 grown alone: *A*, section of incipient perithecium showing the trichogyne-like receptive hypha from the base of the ascogonium; *B*, trichogyne tip end above and outside the young perithecium, the branched basal part of ascogonium in lower part; *C*, *D*, two adjacent sections of two incipient perithecia; at the right in *C*, a receptive hypha, the only one emerging from this perithecial fundament; the series of sections of the perithecium at the left show no emerging receptive hyphae; *E*, trichogyne growing around in the sterile tissue seeking a way out; *F*, a trichogyne emerging at the left and a central cell of the ascogonium sending out another receptive hypha; *G*, trichogyne pushing in between sterile cells on its way to the surface; *H*, tip end of ascogonium here gave rise to two trichogynes, only one showing in this section; the subterminal cell also branched (see plate 38, *F*, for enlarged view of same section); *I*, section showing sterile trichogyne-like hairs arising from the outer cells of the incipient perithecium, having no connection with the ascogonium and can not be receptive; *J*, central cell of ascogonium giving rise to a trichogynous element, not common.

PLATE 38

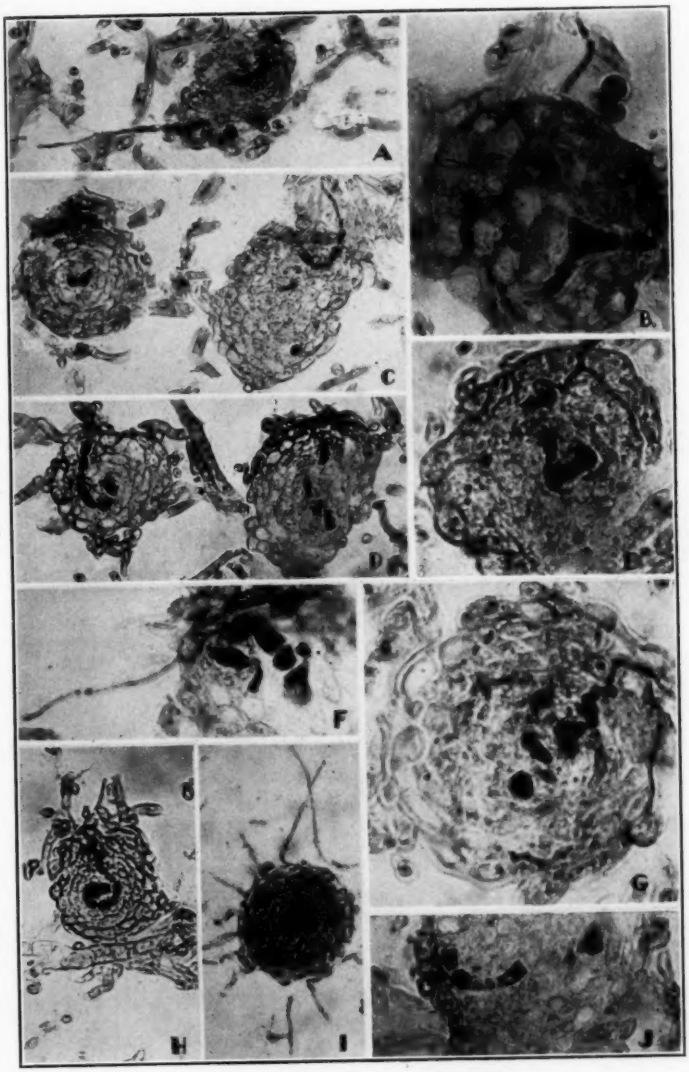
Neurospora sitophila: A, trichogyne-like receptive hypha, probably arising from a basal cell of the ascogonium; *B*, hypha extending out through the base of the perithecium is not a trichogyne, but may well have been receptive; *C*, branched receptive hypha from basal cell of ascogonium, next section showed the connection between this branch and the rest of the ascogonium; *D*, photograph, inverted, of receptive hypha from base of ascogonium, no trichogyne from apical end; *E*, basal cell branched to give rise to two receptive hyphae, the ascogonium branched again above (see *H* for the next section of this incipient perithecium, photograph mounted at 90°, shows either another branch from the same basal cell or the other half one of those shown in *E*); *F*, more highly magnified section of same body shown in plate 37, *H*; *G*, open cytoplasmic connection between basal cell of ascogonium and the branched receptive hypha which was early surrounded by sterile tissue, no trichogyne found in the series of sections; *H*, see legend for *E*; *I*, coiled ascogonium showing basal cell branched, incipient perithecia often inverted.

PLATE 39

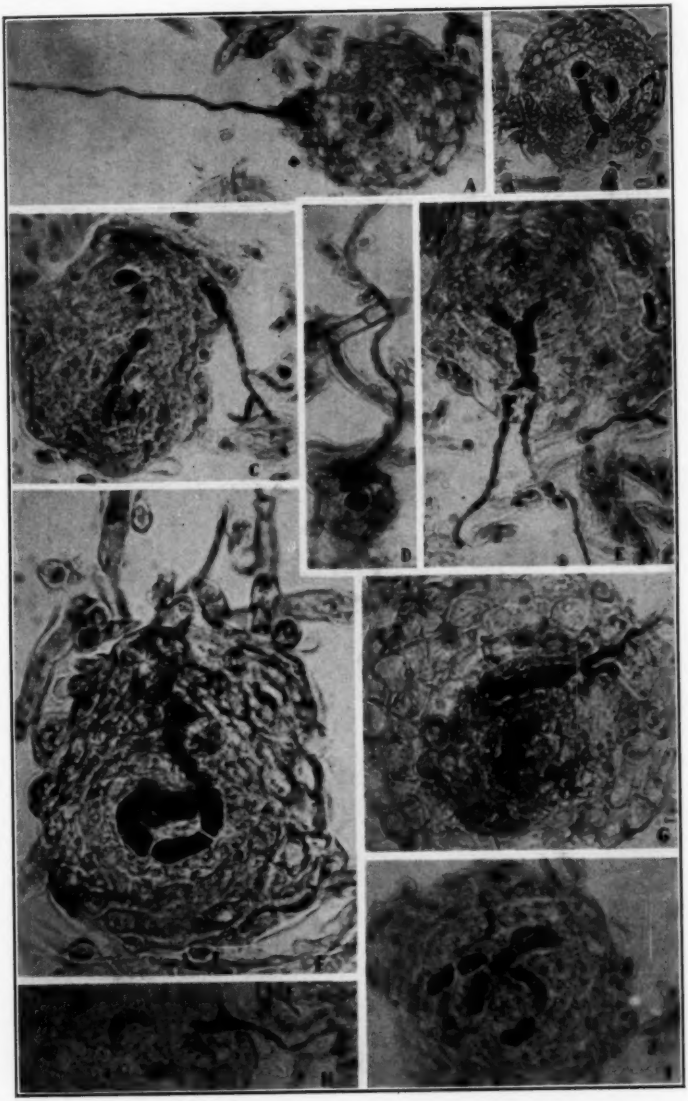
A, plate culture of *Gelasinospora tetrasperma* showing perithecial distribution pattern which is exactly like that illustrated by Dowding (**13**) and is the same type of pattern one obtains in many matings of *Neurospora tetrasperma* referred to in our text (p. 421); *B*, tube cultures of *Neurospora tetrasperma* in which the top of the slant was inoculated with a sex A strain in each case; the number at the left on each tube refers to the sex A race, and the one at the right to the particular sex B race used; perithecia mostly formed on the sex A mycelia. (These cultures were kept several months without any material change in the pattern except the addition of numbers of perithecia down in the agar but always on the A mycelium.)

PLATE 40

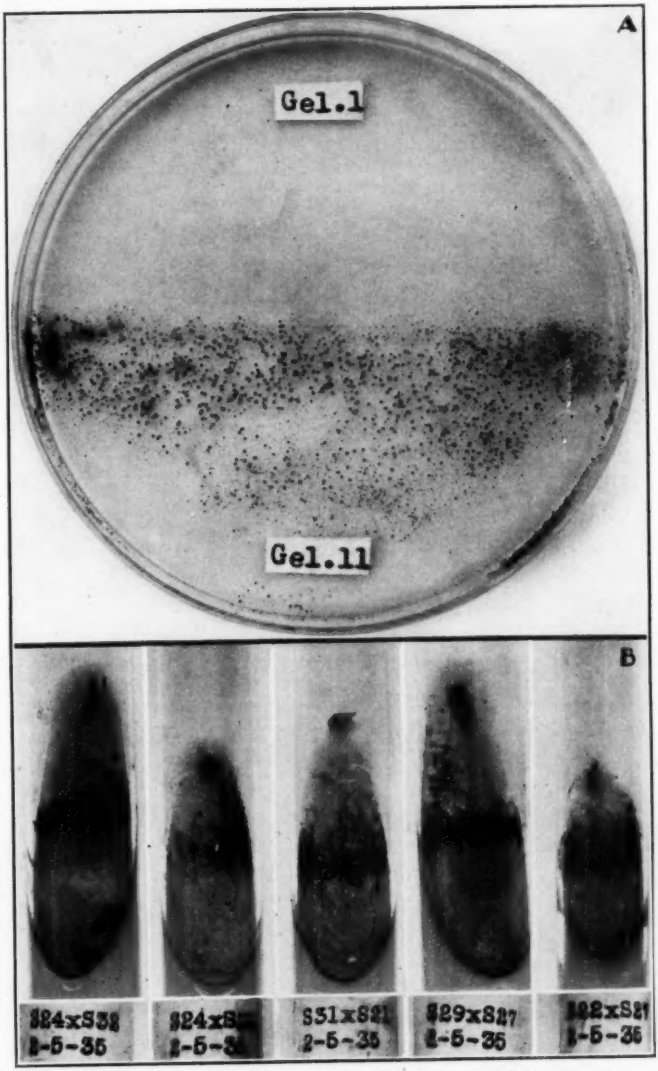
A, plate culture showing odd distribution pattern in which nuclear migration was not uniform; the streaks of perithecia down along lines of growth showing where nuclei from S33, which is sex B in reaction, moved down along the S22, sex A, hyphae; *B*, S4 is sex A, and S27 is sex B in reaction; the first perithecia formed were those scattered on the S27 side; the dense zone of ascocarps showing that heterokaryosis was very complete in this region, nuclear migration from S27 down S22 hyphae very slow. Nuclear migration toward the S27 side also.



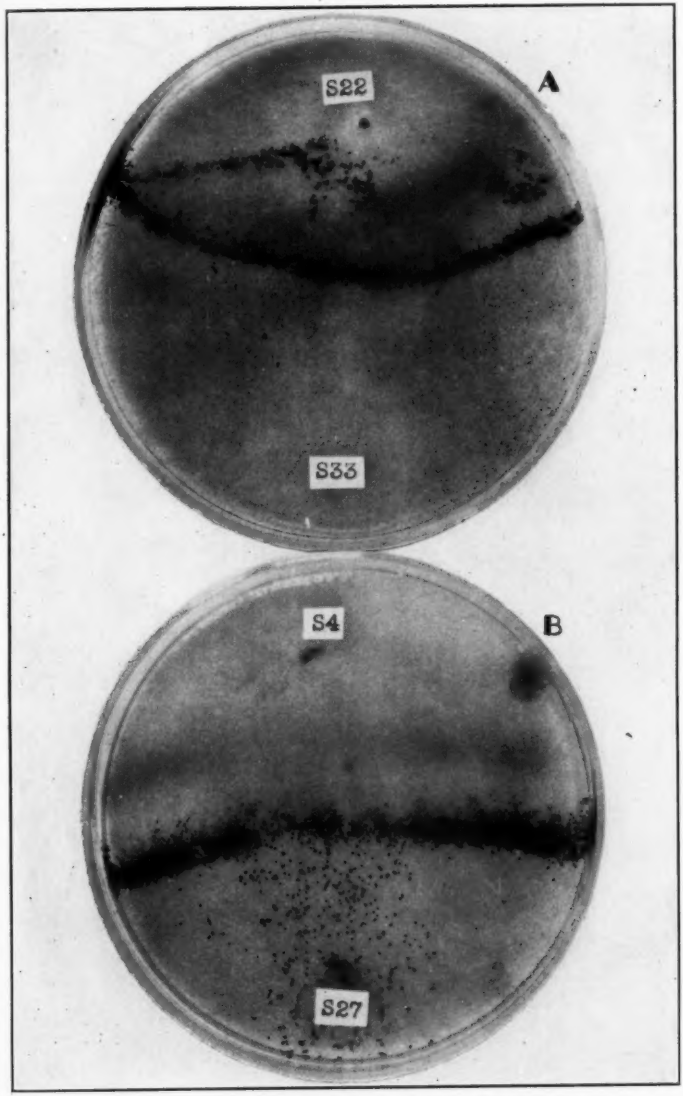
INCIPIENT PERITHECIA OF NEUROSPORA



INCIPIENT PERITHECIA OF NEUROSPORA



DISTRIBUTION PATTERNS OF GELASINOSPORA AND NEUROSPORA



DISTRIBUTION PATTERNS OF NEUROSPORA